(54) Title: OLIGOMERS THAT BIND TO KU PROTEIN
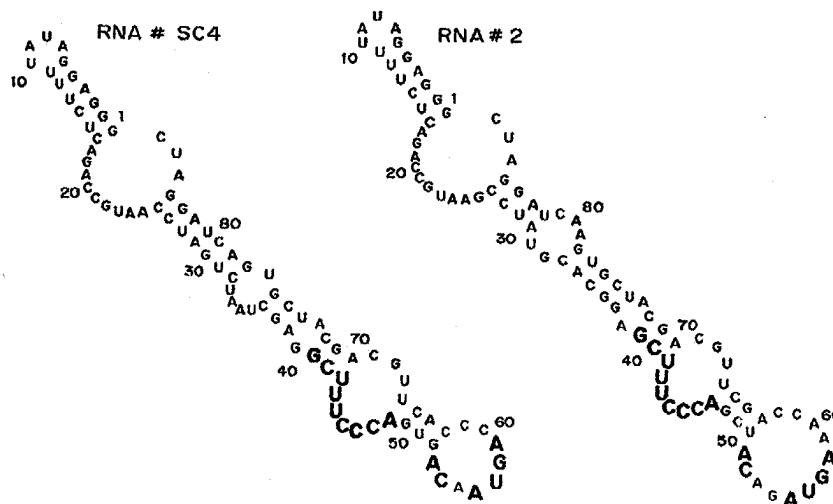
(57) Abstract

    Disclosed are oligomers that bind Ku protein. These oligomers are useful for inhibiting activation of DNA–PK, treating certain forms of autoimmune disease, detection and purification of Ku protein, and identification of proteins that interact with Ku protein. Preferably, the oligomers are composed of nucleotides, nucleotide analogs, or a combination. Most preferably, the oligomers are composed of ribonucleotides. Also disclosed is a method of inhibiting DNA repair, a method of identifying cellular proteins that interact with Ku protein, and a method of treating autoimmune disease in patients with anti–Ku antibodies. The disclosed oligomers can have several preferred features, either alone or in combination, in addition to Ku binding. One such feature, referred to herein as inhibition activity, is inhibition DNA–PK kinase activity. Another preferred feature, referred to herein as aptamer motifs, is the presence of one or more of the base sequences GCUUUCCCANNNAC, A(A/C)AUGA, and AACUUCGA.

OLIGOMERS THAT BIND TO KU PROTEIN

Field of the Invention

The present invention is generally in the area of oligomers, and more
specifically in the area of nucleotide sequences that block the function of

5   DNA-dependent protein kinase.

Statement Regarding Federally Funded Research

10   **Background of the Invention**

Ku protein, a heterodimer of 70 kDa and 83 kDa polypeptides, is the
regulatory component of the DNA-dependent protein kinase (DNA-PK). Ku
protein binds to DNA discontinuities and is essential for DNA double-strand
break repair.

15   Ku protein was first identified as an autoantigen in sera from certain
patients with autoimmune disease (Mimori et al., J. Biol. Chem. 261, 2274-
2278 (1986)). Subsequent characterization showed that Ku protein binds
avidly to double-stranded DNA ends and other structural discontinuities in
DNA such as nicks, gaps, and hairpins (Mimori & Hardin, J. Biol. Chem.

20   261, 10375-10379 (1986); Paillard & Strauss, Nucleic Acids Res. 19, 5619-
5624 (1991); Zhang & Yaneva, Biochem. Biophys. Res. Commun. 186, 574-
479 (1992); Blier et al., J. Biol. Chem. 268, 7594-7601 (1993); Falzon et al.,
J. Biol. Chem. 268, 10546-10552 (1993)). Further biochemical analysis
demonstrated that Ku protein is the regulatory component of the DNA-

25   dependent protein kinase (Dvir et al., Proc. Natl. Acad. Sci. USA 89, 11920-
11924 (1992); Gottlieb & Jackson, Cell 72, 131-142 (1993)). In the presence
of DNA ends, Ku protein can interact with the catalytic subunit of DNA-PK
(DNA-PKcs) which is thereby targeted to the DNA. The ability of Ku
protein to interact with DNA ends suggested that Ku and DNA-PKcs may

30   play a role in DNA repair and recombination (Anderson, Trends Biochem.
Sci. 18, 433-437 (1993)). Subsequent characterization of ionizing radiation-
sensitive mutant cell lines showed that Ku protein and DNA-PKcs are
essential for repair of DNA double-strand breaks and for V(D)J

recombination (Getts & Stamato, J. Biol. Chem. 269, 15981-15984 (1994);
Rathmell, Proc. Natl. Acad. Sci. USA 91, 7623-7627 (1994); Smider et al.,
Science 266, 288-291 (1994); Taccioli et al., Science 265, 1442-1445 (1994);
Blunt et al., Cell 80, 813-823 (1995); Boubnov et al., Proc. Natl. Acad. Sci.

5   USA 92, 890-894 (1995); Kirchgessner et al., Science 267, 1178-1183
(1995); Lees-Miller et al., Science 267, 1183-1185 (1995); Peterson et al.,
Proc. Natl. Acad. Sci. USA 92, 3171-3174 (1995)).

The binding of Ku protein to double-stranded DNA ends is largely
sequence-independent. The ability of Ku protein to undergo facilitated

10   transfer between DNA fragments with cohesive ends suggests that Ku
protein may be able to interact transiently with two DNAs simultaneously,
perhaps serving to align the ends for ligation (Bliss & Lane, J. Biol. Chem.
272, 5765-5773 (1997)). Consistent with this, recent atomic force
microscopy and electron microscopy studies show images of Ku protein

15   tethering DNA fragments together and participating in loop structures (Cary,
Proc. Natl. Acad. Sci. USA 94, 4267-4272 (1997); Pang et al., Cancer Res.
57, 1412-1415 (1997)). There have also been a number of reports of possible
sequence-specific binding of Ku protein to DNA (for example, Knuth et al.,
J. Biol. Chem. 265, 17911-17920 (1990); Messier et al., Proc. Natl. Acad.

20   Sci. USA 90, 2685-2689 (1993); Okumura et al., FEBS Lett. 356, 94-100
(1994); Roberts et al., Proc. Natl. Acad. Sci., USA 91, 6354-6358 (1994)).
Most recently, a sequence in the long terminal repeat of mouse mammary
tumor virus has been characterized that appears to allow interaction of Ku
protein with DNA in the absence of ends or single-stranded regions (Giffin et

25   al., Nature 380, 265-268 (1996); Giffin et al., J. Biol. Chem. 272, 5647-5658
(1997)).

There is some evidence that Ku protein interacts with RNA, although
this has been much less studied than the interaction with DNA. Antibodies
to Ku protein stain both the nucleoplasm and the nucleolus. The amount of

30   Ku protein in the nucleolus changes depending on the growth state of the
cell, suggesting that this localization is actively regulated (Yaneva & Jhiang,
Biochim. Biophys. Acta 1090, 181-187 (1991)). Separately, it has been
demonstrated that nucleolar staining is sensitive to RNase treatment, whereas

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nucleoplasmic staining is not (Reeves, J. Exp. Med. 161, 18-39 (1985)).
Thus, nucleolar localization may be regulated by interaction of Ku protein
with RNA. Ku protein does not appear to bind to bulk tRNA or to synthetic
RNA polymers (Mimori & Hardin, J. Biol. Chem. 261, 10375-10379

5   (1986)). However, one study showed that Ku protein forms a specific
complex with an RNA that included the HIV trans-activation response
(TAR) element sequence (Kaczmarski & Khan, Biochem. Biophys. Res.
Commun. 196, 935-942 (1993)).

      The Ku protein has been suggested to be involved in many important

10   nuclear processes, including transcription, replication, and growth control, as
well as DNA repair. It would be useful to have a means to demonstrate
whether Ku protein is required for a biochemical activity (such as repair or
recombination) that has been reconstituted in a crude cell-free system. It
would also be useful to have a means to identify nuclear proteins that

15   physically interact with Ku protein to exert their biological functions.

      Ku protein and DNA-PK are important in the repair of radiation-
induced DNA damage. If damage cannot be repaired, cells die. The
cytotoxic effect of ionizing radiation forms the basis for radiation therapy,
which is widely used in the treatment of human cancer. The efficacy of

20   radiation therapy is currently limited by the radiation resistance of certain
tumors (for example, glioblastomas) and by the side effects caused by
irradiation of nearby normal tissues (for example, in treatment of breast and
cervical cancer). Therefore, it would also be useful to have a means for
sensitizing target cells and tissues to therapeutic radiation.

25   Some patients with autoimmune diseases such as systemic lupus
erythematosus (SLE) and scleroderma produce anti-Ku antibodies. Ku is one
of a number of proteins that are targets of autoantibodies in these patients.
High levels of autoantibodies lead to deleterious consequences for the
patient. It would be useful to have a compound directed against Ku protein

30   to alter the course of autoimmune disease in patients with anti-Ku antibodies.

      A problem in gene therapy is a loss of the foreign DNA from
illegitimate recombination. It is believed that illegitimate recombination

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requires Ku-dependent double strand break repair. It would be useful to have a means to improve the stability of transgene DNA.

Therefore, it is an object of the disclosed invention to provide oligomers that bind to Ku protein.

5   It is also an object of the disclosed invention to provide oligomers that prevent illegitimate recombination to improve the stability of transgene DNA.

It is another object of the disclosed invention to provide oligomers that recognize Ku protein in a complex environment containing other

10   macromolecules.

It is also an object of the disclosed invention to provide oligomers that improve the efficacy of radiation therapy by inhibiting DNA repair in the target cells.

It is also an object of the disclosed invention to provide oligomers to

15   treat autoimmune disease in patients with anti-Ku antibodies.

It is also an object of the disclosed invention is to provide an assay for cellular proteins that interact with Ku protein or that promote or inhibit the interaction between Ku protein and DNA-PKcs.

It is also an object of the disclosed invention is to provide oligomers

20   useful for manipulating the activity of Ku protein in cells and organisms to better understand its physiological role.

Summary of the Invention

Disclosed are oligomers that bind Ku protein. These oligomers, also referred to herein as aptamers, are useful for inhibiting activation of DNA-

25   PK, treating certain forms of autoimmune disease, detection and purification of Ku protein, and identification of proteins that interact with Ku protein. Preferably, the oligomers are composed of nucleotides, nucleotide analogs, or a combination. Most preferably, the oligomers are composed of ribonucleotides. Also disclosed is a method of inhibiting DNA repair, a

30   method of identifying cellular proteins that interact with Ku protein, and a method of treating autoimmune disease in patients with anti-Ku antibodies.

The disclosed oligomers can have several preferred features, either alone or in combination, in addition to Ku binding. One such feature,

referred to herein as inhibition activity, is inhibition of DNA-PK kinase

activity. As discussed above, interaction of the Ku protein and DNA is

involved in activation of DNA-PK kinase activity. Another preferred

feature, referred to herein as aptamer motifs, is the presence of one or more

5   of the base sequences GCUUUCCCANNNAC, A(A/C)AUGA, and

AACUUCGA. These sequences -- referred to herein as aptamer motif 1,

aptamer motif 2, and aptamer motif 3, respectively -- are associated with Ku

binding capability. Another preferred feature, referred to herein as aptamer

structure, is the presence of a structure similar to the structure shown in

10   Figure 6A. This structure has the general formula 5'-A-B-C-D-C'-E-A'-3',

where A, B, C, D, C', E, and A' are components of the oligomer. In this

structure, A and A' interact to form a stem structure, C and C' interact to

form a stem structure, B and E make up a bulge region, and D is either a

bulge or a loop. Figure 6A depicts component D as a loop. Each of these

15   preferred features (inhibition activity, aptamer motif, and aptamer structure)

can be used either alone or in combination with one or both of the other

characteristics.

Brief Description of the Drawings

Figure 1 is a graph of percent RNA or DNA bound versus

20   concentration of Ku (nM) for five representative Ku protein-RNA and DNA

binding curves obtained with an electrophoretic mobility shift assay. The

graph shows that pooled RNA tested after the fourth and sixth round of

Systematic Evolution of Ligands by Exponential Enrichment (SELEX; Tuerk

& Gold, Science 249:505-510 (1990), and U.S. Patent Numbers 5,270,163

25   and 5,567,588) had an increased ability to bind to Ku protein. Radiolabeled

nucleic acid probes were as follows: □, nonselected RNA; ▽, RNA after 4th

round of selection; ▲, RNA after 6th round of selection; O, HIV-TAR RNA

(included for comparison); ◆, 21 base pair double-stranded DNA.

Figure 2A is a graph of kinase activity (percentage of activity in

30   absence of RNA) versus RNA concentration (nM) for eight representative

aptamers. The aptamers were as follows: X, nonselected RNA; O, #2; □,

#7-3; △, SC4; +, SC5; ◆, SC8; ▼, #1-2; ●, SC2.

Figure 2B is a graph of DNA binding (percent of Ku-DNA complexes detected in the absence of competitor) versus RNA concentration (nM) for seven representative aptamers. Purified Ku protein (2 nM) was incubated with radiolabeled 21 base pair double-stranded DNA (1 nM) in the

5 presence of various amounts of nonselected RNA or aptamer. The aptamers were as follows: X, nonselected RNA; O, #2; □, #7-3; △, SC4; +, SC5; ♦, SC8; ▼, #1-2; ●, SC2.

Figure 3 is a bar graph of kinase activity (percentage of activity in the absence of RNA) in the presence of different aptamers, each at three

10 different concentrations. Reactions were performed in crude nuclear extract. Reactions contained 2 µl HeLa cell nuclear extract (0.4 µg protein), substrate (Ku protein (2 nM)) and DNA (1 nM) were as in Figure 2. Reactions were performed in the presence of various amounts of aptamer (250, 500, 1000 nM).

15 Figure 4 is the predicted secondary structure of examples of the disclosed aptamers, SC4 and #2. RNA secondary structures were predicted using the methods of Zuker and co-workers (Jaeger et al., 1989; Jaeger et al., 1990) as implemented on the mfold World Wide Web server (www.ibc.wustl.edu/zuker/rna). Boldface indicates the conserved sequence

20 motifs as shown in Table 1.

Figure 5 shows the predicted secondary structure of the aptamer SC4 and the predicted structures of the mutated aptamers, SC4/D1 and SC4/D2. The 3' invariant region of the aptamer, i.e. the twenty-three 3' terminal bases, was removed from the SC4/D1 aptamer and an additional 15 bases

25 were removed from the 3' end of the molecule in the SC4/D2 aptamer.

Figure 6A is a representation of the general secondary structure for a preferred form of aptamer.

Figure 6B is a comparison of the predicted secondary structure of a preferred form of aptamer with the secondary structure of a hypothetical

30 double-strand DNA break repair intermediate.

Detailed Description of the Invention

Disclosed are oligomers that bind Ku protein. It is preferred that the oligomers bind Ku protein with a dissociation constant of less than 10 nM.

These oligomers are useful for inhibiting activation of DNA-PK, treating
certain forms of autoimmune disease, detection and purification of Ku
protein, and identification of proteins that interact with Ku protein.
Preferably, the oligomers are composed of nucleotides, nucleotide analogs,
5   or a combination. Most preferably, the oligomers are composed of
ribonucleotides. Also disclosed is a method of inhibiting DNA repair, a
method of identifying cellular proteins that interact with Ku protein, and a
method of treating autoimmune disease in patients with anti-Ku antibodies.

The disclosed oligomers can have several preferred features, either
10   alone or in combination, in addition to Ku binding. One such feature,
referred to herein as inhibition activity, is inhibition of DNA-PK kinase
activity. As discussed above, interaction of the Ku protein and DNA is
involved in activation of DNA-PK kinase activity. Another preferred
feature, referred to herein as aptamer motifs, is the presence of one or more
15   of the base sequences GCUUUCCCANNNAC, A(A/C)AUGA, and
AACUUCGA. These sequences -- referred to herein as aptamer motif 1,
aptamer motif 2, and aptamer motif 3, respectively -- are associated with Ku
binding capability. Another preferred feature, referred to herein as aptamer
structure, is the presence of a structure similar to the structure shown in
20   Figure 6A. This structure has the general formula 5'-A-B-C-D-C'-E-A'-3',
where A, B, C, D, C', E, and A' are components of the oligomer. In this
structure, A and A' interact to form a stem structure, C and C' interact to
form a stem structure, B and E make up a bulge region, and D is either a
bulge or a loop. Figure 6A depicts component D as a loop. Each of these
25   preferred features (inhibition activity, aptamer motif, and aptamer structure)
can be used either alone or in combination with one or both of the other
characteristics.

Examples of preferred oligomers are listed in Table 1. These
oligoribonucleotides were identified as described in the examples. Only the
30   portion of the selected oligomers that arose from the variable region of the
pool RNA is shown in Table 1. That is, each of the oligonucleotides listed in
Table 1 include the sequence GGGAGGAUAUUUUCUCAGACCGUAA at
the 5' end and the sequence UUGCAGCAUCGUGAACUAGGAUC at the

3' end, although these sequences are not shown in Table 1. Thus, oligomer

SC6 (SEQ ID NO:1) has the sequence GGGAGGAUAUUUUCUCAGA

CCGUAAGACUCACGAUGGACCAUACGCCUUCCCACUGGUCUUGU

UAUUGCAGCAUCGUGAACUAGGAUC, although only the sequence

5   GACUCACGAUGGACCAUACGCCUUCC CACUGGUCUUGUUA is

shown in the table.

Table 1 also discloses the dissociation constants (Kd) of the

individual aptamers and the ability of the individual aptamers to inhibit

DNA-PK activity. In a standard reaction, aptamers with a DNA-PK activity

10   denoted as A had more than 85% inhibition, aptamers denoted as B had

between 30 and 80% inhibition, and aptamers denoted with a C had under

30% inhibition.

A.   Oligomers

Some embodiments of the oligomers can be described generally by

15   an oligonucleotide comprising the components A, B, C, D, C', E, and A'.

Component A is the 5' most component and component A' is the 3' most

component of the Ku binding oligonucleotides. Components A and A'

interact in such a way as to form a stem structure through nucleotide base

pairing. It is not required that all of the base pairs be contiguous, but it is

20   recognized that those of skill in the art understand that the stability of any

given double stranded nucleic acid molecule is a function of both the number

of base pairs present and the number of bulges or mismatches that are

present. A preferred embodiment has a stem that has a level of stability

equivalent to two C:G base pairs. Another preferred embodiment has a

25   purine:purine base pair, three base pairs from the bulge region comprised by

components B and E (see Figure 4).

Component B represents a bulge structure contiguous and 3' to

component A. Furthermore, this component is preferably composed of

pyrimidines. Most preferably this component contains the sequence 5'-

30   UUUCCC-3' or 5'-UUUCCCA-3'.

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Table 1: RNA Sequences and K$_d$ values from the SELEX Procedure

Name Frequency	Aligned sequence	DNA-PK Inhibition	K$_d$	
CLASS I				
SC6 (3)	GACUCACGAUGGACCAUACGCCUUCCACUGGUCUUGUUA (SEQ ID NO:1)	C	2.0	nM
#1-2 (1)	CAACACCUUGCUUUCCAAUACCUGAAAUACAGUCGGAU (SEQ ID NO:2)	A	1.5	nM
#1-17 (1)	UCCUUAUUUUAUGGCUUUCCACGCACAAGGUCUGCG (SEQ ID NO:3)	B	3.2	nM
#85 (1)	CAAGUAUCACGCACUUUCCAUUCACUGUUAGAGACUGA (SEQ ID NO:4)	B	0.7	nM
HYBRID				
#2 (1)	GCCUAUGCACGGAGCUUUCCCAGCUACAGAUGAAACCAGC (SEQ ID NO:5)	A	0.3	nM
SC4 (8)	CCUAGUCUAAUCGAGGCUUUCCCAGUGAC_AUUGACCCAC (SEQ ID NO:6)	A	1.7	nM
SC5 (4)	CUUGAACAUGAUAG_GCUUACCCAUAGACACAGAUUGACCCUU (SEQ ID NO:7)	A	2.0	nM
CLASS II				
SC9 (3)	UGCCUUUAGCUGCGAC_AAUGAACAGCAUGACCUCACUAC (SEQ ID NO:8)	B	0.6	nM
SC8 (8)	(SEQ ID NO:9) GUCCUUCACUAAUGCUUACCAGCACACUAAGAACGUCAC	A	0.6	nM
SC3 (3)	CAUUACCACAGUUCUAGCAUCCCGCAAUGGUAAGUCCGCA (SEQ ID NO:10)	B	0.8	nM
SC1 (23)	UUGUUCAACCUUGUCUAACAUGAUACCGAUACGGACUACA (SEQ ID NO:11)	B	1.2	nM
#84 (1)	AUCCGCGUACGGGCUCAAAUGUCACUAUAGUAGAAAGCA (SEQ ID NO:12)	B	1.8	nM
#52 (1)	(SEQ ID NO:13) CUGAUCGUUCAAUGACUAUUCUUUACCUUGAGUAACCGA	C	3.2	nM
HYBRID				
SC12 (4)	(SEQ ID NO:14) CUCGCAACAUGACUUCGAAAGUUUAAUCGUUCUUGUCAA	B	0.5	nM
CLASS III				
#7-3 (1)	AGGUCGGCAUACAGAGUUCCGAAUGCGGCUAACUUCGACU (SEQ ID NO:15)	A	1.8	nM
SC11 (2)	(SEQ ID NO:16) CUUAGUUUCGAUCGGAAUGCGAUUAACUUACGCUUCAUA	B	>10	nM
SC2 (12)	CACGCUCUACAACAGAUUGCGAAUUAACUUACGCUUCAUA (SEQ ID NO:17)	A	0.8	nM
#42 (1)	CAUCCUGGUACUCACUUCGACAUCGUACGUUCAAUCAUAC (SEQ ID NO:18)	B	4.5	nM
OTHER				
SC13 (3)	ACCUUUUAGACGAACCUCAAAGUACAUUUAGUUGAAAAC (SEQ ID NO:19)	B	0.8	nM

RNA sequences are aligned to show maximum sequence identity. The underlined sequence indicates sequence identity within class.

Component C is contiguous and 3' to component B. Components C and C' form a stem region between the bulge formed by components B and E and the loop region D. It is also understood that this region can contain mismatches and bulges but that a certain level of stability is required. In preferred embodiments this stem will possess a stability equivalent to a stem made up of an AGCU:UCGA stem.

Component D is contiguous and 3' to component C. Component C' is contiguous and 3'- to component D. Component D is a sequence which preferably forms a loop structure. In a preferred embodiment component D contains the sequence 5'-ACNNAUGA-3'. In another preferred embodiment component D contains the sequence 5'-ACNNAUGANNNN-3'. In another preferred embodiment component D contains 5'-ACMAUGANNNN-3' (where M is A or C). In a most preferred embodiment Component D contains the sequence 5'-ACAGAUGAAACC-3'. It is also recognized that this loop structure can also be represented by a bulge-stem structure. Those in the art will recognize how to turn the loop structure into a bulge-stem structure. This would be done for example if there were two oligomers that were used to form the preferred structure.

Component E is contiguous and 3' to component C'. Component E is a bulged region. In the most preferred embodiment component B contains the sequence 5'-UGC-3'.

1. Oligomers Containing Chemically Modified Nucleotides.

RNA is relatively labile and can be degraded by a number of ribonucleases. This degradation can be greatly reduced by the introduction of modifications and substitutions at the 2'-prime position of the ribonucleotide and by modifications and substitutions along the phosphate backbone of the RNA. In addition a variety of modifications can be made on the nucleobases themselves which both inhibit degradation and which can increase desired nucleotide interactions or decrease undesired nucleotide interactions. Once the sequence of an aptamer is known modifications or substitutions can be made by the synthetic procedures described below or by

procedures known to those of skill in the art. Aptamers with these various modifications can then be tested for function using any suitable assay for the Ku function of interest, such as the Ku protein binding assay or the inhibition of DNA-PK activity assay.

5   The oligomers disclosed are made up of nucleotides and/or nucleotide analogs or a combination of both, or are oligonucleotide analogues. The disclosed oligomers described by the general structure above may contain nucleotide analogs at positions which do not effect the function of the oligomer to bind Ku protein. The oligomeric sequences specifically interact

10   with Ku protein, preferably at the DNA binding site of Ku protein.

        Components A, A', B, C, C', D, and E are made up of nucleotides and nucleotide analogues in which the nucleotide and nucleotide analogs have the structure

15
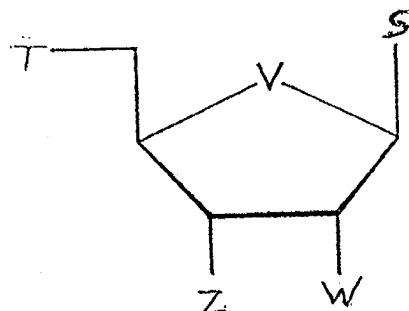
(I)

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25   In structure (I) each S can be adenin-9-yl, cytosin-1-yl, guanin-9-yl, uracil-1-yl, uracil-5-yl, hypoxanthin-9-yl, thymin-1-yl, 5-methylcytosin-1-yl, 2,6-diaminopurin-9-yl, purin-9-yl, 7-deazaadenin-9-yl, 7-deazaguanin-9-yl, 5-propynylcytosin-1-yl, 5-propynyluracil-1-yl, isoguanin-9-yl, 2-aminopurin-9-yl, 6-methyluracil-1-yl, 4-thiouracil-1-yl, 2-pyrimidone-1-yl,

30   quinazoline-2,4-dione-1-yl, xanthin-9-yl, N^2-dimethylguanin-9-yl or a functional equivalent thereof;

        Each V can be an O, S, NH, or CH_2 group.

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Each W can be -H, -OH, -COOH, -$CONH_2$, -$CONHR^1$, -$CONR^1R^2$, -NH_2, -NHR^1, -NR^1R^2, -$NHCOR^1$, -SH, SR^1, -F, -ONH_2, -$ONHR^1$, -ONR^1R^2, -NHOH, -$NHOR^1$, -NR^2OH, -NR^2OR^1, substituted or unsubstituted C_1-C_{10} straight chain or branched alkyl, substituted or

5   unsubstituted C_2-C_{10} straight chain or branched alkenyl, substituted or unsubstituted C_2-C_{10} straight chain or branched alkynyl, substituted or unsubstituted C_1-C_{10} straight chain or branched alkoxy, substituted or unsubstituted C_2-C_{10} straight chain or branched alkenyloxy, and substituted or unsubstituted C_2-C_{10} straight chain or branched alkynyloxy. The

10   substituents for W groups are independently halogen, cyano, amino, carboxy, ester, ether, carboxamide, hydroxy, or mercapto. R^1 and R^2 can be substituted or unsubstituted alkyl, alkenyl, or alkynyl groups, where the substituents are independently halogen, cyano, amino, carboxy, ester, ether, carboxamide, hydroxy, or mercapto.

15   T and Z are residues which together form a phosphodiester or phosphorothioate diester bond between adjacent nucleosides or nucleoside analogues or together form an analogue of an internucleosidic bond.

The 3' end of the disclosed oligomers can be protected against degradation by exonucleases by, for example, using a nucleotide analogue

20   that is modified at the 3' position of the ribose sugar (for example, by including a substituted or unsubstituted alkyl, alkoxy, alkenyl, alkenyloxy, alkynyl or alkynyloxy group as defined above). The disclosed oligomers can also be stabilized against degradation at the 3' end by exonucleases by including a 3'-3'-linked dinucleotide structure (Ortigao *et al.*, *Antisense*

25   *Research and Development* 2:129-146 (1992)) and/or two modified phospho bonds, such as two phosphorothioate bonds.

The disclosed oligomers can also be linked to a prosthetic group in order to improve their cellular uptake and/or to enable a specific cellular localization. Examples of such prosthetic groups are polyamino acids (for

30   example, polylysine), lipids, hormones or peptides. These prosthetic groups are usually linked via the 3' or 5' end of the oligomer either directly or by means of suitable linkers (for example, linkers based on 6-aminohexanol or

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6-mercaptohexanol). These linkers are commercially available and techniques suitable for linking prosthetic groups to the oligomer are known to a person skilled in the art. The oligomers can also be produced as fusion oligonucleotides as discussed below.

5   In preferred oligomers, the oligonucleotides are made from RNA. The examples of Ku aptamers obtained as described in the examples are in the form of RNA. These RNAs were selected from a pool of about 10^{14} different RNA sequences using the systematic evolution of ligands by exponential enrichment (SELEX) procedure. Most of the selected RNAs

10   bind to Ku protein with an equilibrium dissociation constant (K_d) ≤ 2 nM, comparable to the affinity of DNA fragments for Ku protein under similar conditions. Many of the RNAs inhibited DNA-PK activity up to 85% by competing with DNA for a common binding site in Ku protein. None of the several RNAs tested activated DNA-PK in the absence of DNA.

15   **2.   Isolating RNAs That Bind Ku Protein.**

There are several techniques that can be adapted for refinement of the disclosed aptamers or the selection of additional aptamers. Preferred are the techniques described in the examples. Other techniques can also be used. These include techniques generally referred to as "in vitro genetics"

20   (Szostak, TIBS 19:89, 1992).

Molecules that inhibit or activate Ku mediated DNA-PK function can be isolated by screening for molecules that inhibit function of DNA-PK without inhibiting the interaction of DNA-PK with Ku protein. For example, molecules that inhibit DNA binding to DNA-PK may inhibit DNA-PK

25   function without inhibiting DNA-PK:Ku interaction.

The disclosed aptamers can also be refined through the use of computer modeling techniques. Examples of molecular modeling systems are the CHARMm and QUANTA programs, Polygen Corporation, Waltham, MA. CHARMm performs the energy minimization and molecular dynamics

30   functions. QUANTA performs the construction, graphic modeling and analysis of molecular structure. QUANTA allows interactive construction, modification, visualization, and analysis of the behavior of molecules with

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each other. These applications can be adapted to define and display the secondary structure of RNA and DNA molecules.

The pool of nucleic acid molecules from which the disclosed aptamers were isolated included invariant sequences flanking a variable

5   sequence of approximately forty nucleotides. The 5' invariant region contained 27 nucleotides and the 3' invariant region contained 25 nucleotides. The invariant regions were used for the amplification of the molecules that were enriched through the SELEX procedure described below. This pool was chosen because it had been shown to function well in

10   SELEX protocols. (Chen and Gold, Biochemistry 33, 8746-8756 (1994)).

3.   Synthesis of Oligomers.

The disclosed oligomers can be synthesized using any suitable method. Many synthesis methods are known. The following techniques are preferred for synthesis of for example, 2'-O-Allyl modified oligomers that

15   contain residual purine ribonucleotides, and bearing a suitable 3'-terminus such as an inverted thymidine residue (Ortigao et al., Antisense Research and Development 2:129-146 (1992)) or two phosphorothioate linkages at the 3'-terminus to prevent eventual degradation by 3'-exonucleases, can be synthesized by solid phase β-cyanoethyl phosphoramidite chemistry (Sinha

20   et al., Nucleic Acids Res. 12:4539-4557 (1984)) on any commercially available DNA/RNA synthesizer. A preferred method is the 2'-O-tert-butyldimethylsilyl (TBDMS) protection strategy for the ribonucleotides (Usman et al., J. Am. Chem. Soc. 109:7845-7854 (1987)), and all the required 3'-O-phosphoramidites are commercially available. In addition, the

25   use of aminomethylpolystyrene is preferred as the support material due to its advantageous properties (McCollum and Andrus Tetrahedron Letters 32:4069-4072 (1991)). Fluorescein can be added to the 5'-end of a substrate RNA during the synthesis by using commercially available fluorescein phosphoramidites. In general, a desired oligomer can be synthesized using a

30   standard RNA cycle. Upon completion of the assembly, all base labile protecting groups are removed by an 8 hour treatment at 55°C with concentrated aqueous ammonia/ethanol (3:1 v/v) in a sealed vial. The

ethanol suppresses premature removal of the 2'-O-TBDMS groups which would otherwise lead to appreciable strand cleavage at the resulting ribonucleotide positions under the basic conditions of the deprotection (Usman *et al., J. Am. Chem. Soc.* 109:7845-7854 (1987)). After

5   lyophilization the TBDMS protected oligomer is treated with a mixture of triethylamine trihydrofluoride/triethylamine/N-methylpyrrolidinone for 2 hours at 60°C to afford fast and efficient removal of the silyl protecting groups under neutral conditions (Wincott *et al., Nucleic Acids Res.* 23:2677- 2684 (1995)). The fully deprotected oligomer can then be precipitated with

10   butanol according to the procedure of Cathala and Brunel (*Nucleic Acids Res.* 18:201 (1990)). Purification can be performed either by denaturing polyacrylamide gel electrophoresis or by a combination of ion-exchange HPLC (Sproat *et al., Nucleosides and Nucleotides* 14:255-273 (1995)) and reversed phase HPLC. For use in cells, it is preferred that synthesized

15   oligomers be converted to their sodium salts by precipitation with sodium perchlorate in acetone. Traces of residual salts are then preferably removed using small disposable gel filtration columns that are commercially available. As a final step it is preferred that the authenticity of the isolated oligomers is checked by matrix assisted laser desorption mass spectrometry (Pieles *et al.,*

20   *Nucleic Acids Res.* 21:3191-3196 (1993)) and by nucleoside base composition analysis. In addition, a functional cleavage test with the oligomer on the corresponding chemically synthesized short oligoribonucleotide substrate is also preferred.

       The disclosed oligomers can also be produced through enzymatic

25   methods, when the nucleotide subunits are available for enzymatic manipulation. For example, the RNA molecules can be made through *in vitro* RNA polymerase T7 reactions. They can also be made by strains of bacteria or cell lines expressing T7, and then subsequently isolated from these cells. As discussed below, the disclosed aptamers can also be

30   expressed in cells directly using vectors and promoters.

B.   Pharmaceutical Compositions.

The disclosed oligomers can be used in pharmaceutical mixtures that contain one or several oligomers as the active substance, and, optionally, pharmaceutically acceptable auxiliary substances, additives and carriers.

5   Such pharmaceutical oligomers are suitable for the production of an agent to specifically inhibit Ku DNA binding activity in cells or inhibit the activity of DNA-PK. The disclosed oligomers can also be used for Ku inhibition and DNA-PK inhibition in plants or animals. Thus, the disclosed oligomers are expected to be useful as drugs for humans and animals as well as a pesticide

10   for plants.

As used herein, microparticles include liposomes, virosomes, microspheres and microcapsules formed of synthetic and/or natural polymers. Methods for making microcapsules and microspheres are known to those skilled in the art and include solvent evaporation, solvent casting, spray drying and solvent

15   extension. Examples of useful polymers which can be incorporated into various microparticles include polysaccharides, polyanhydrides, polyorthoesters, polyhydroxides and proteins and peptides.

Liposomes can be produced by standard methods such as those reported by Kim *et al., Biochim. Biophys. Acta,* 728:339-348 (1983); Liu *et*

20   *al., Biochim. Biophys. Acta,* 1104:95-101 (1992); and Lee *et al., Biochim. Biophys. Acta.,* 1103:185-197 (1992); Wang *et al., Biochem.,* 28:9508-9514 (1989)). Such methods have been used to deliver nucleic acid molecules to the nucleus and cytoplasm of cells of the MOLT-3 leukemia cell line (Thierry and Dritschilo, *Nucl. Acids Res.,* 20:5691-5698 (1992)).

25   Alternatively, the disclosed oligomers can be incorporated within microparticles, or bound to the outside of the microparticles, either ionically or covalently.

Cationic liposomes or microcapsules are microparticles that are particularly useful for delivering negatively charged compounds such as the

30   disclosed compounds, which can bind ionically to the positively charged outer surface of these liposomes. Various cationic liposomes have previously been shown to be very effective at delivering nucleic acids or

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nucleic acid-protein complexes to cells both *in vitro* and *in vivo*, as reported by Felgner *et al., Proc. Natl. Acad. Sci. USA*, 84:7413-7417 (1987); Felgner, *Advanced Drug Delivery Reviews*, 5:163-187 (1990); Clarenc *et al., Anti-Cancer Drug Design*, 8:81-94 (1993). Cationic liposomes or microcapsules

5   can be prepared using mixtures including one or more lipids containing a cationic side group in a sufficient quantity such that the liposomes or microcapsules formed from the mixture possess a net positive charge which will ionically bind negatively charged compounds. Examples of positively charged lipids that may be used to produce, cationic liposomes include the

10   aminolipid dioleoyl phosphatidyl ethanolamine (PE), which possesses a positively charged primary amino head group; phosphatidylcholine (PC), which possess positively charged head groups that are not primary amines; and N[1-(2,3-dioleyloxy)propyl]-N,N,N-triethylammonium ("DOTMA," see Felgner *et al., Proc. Natl. Acad. Sci USA*, 84:7413-7417 (1987); Felgner *et*

15   *al., Nature*, 337:387-388 (1989); Felgner, *Advanced Drug Delivery Reviews*, 5:163-187 (1990)).

         The disclosed oligomers can also be encapsulated by or coated on cationic liposomes which can be injected intravenously into a mammal. This system has been used to introduce DNA into the cells of

20   multiple tissues of adult mice, including endothelium and bone marrow, where hematopoietic cells reside (see, for example, Zhu *et al., Science*, 261:209-211 (1993)).

         C.   Vectors And Expression Sequences

         Delivery and expression of the disclosed oligomers can be facilitated

25   by vectors and expression sequences. As used herein, plasmid or viral vectors are agents that transport the gene into the cell without degradation and include a promoter yielding expression of the gene in the cells into which it is delivered. In a preferred embodiment vectors are derived from either a virus or a retrovirus. Preferred viral vectors are Adenovirus, Adeno-

30   associated virus, Herpes virus, Vaccinia virus, Polio virus, AIDS virus, neuronal trophic virus, Sindbis and other RNA viruses, including these viruses with the HIV backbone. Also preferred are any viral families which

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share the properties of these viruses which make them suitable for use as vectors. Preferred retroviruses include Murine Maloney Leukemia virus, MMLV, and retroviruses that express the desirable properties of MMLV as a vector. Retroviral vectors are able to carry a larger genetic payload, i.e., a

5   transgene or marker gene, than other viral vectors, and for this reason are a commonly used vector. However, they are not useful in non-proliferating cells. Adenovirus vectors are relatively stable and easy to work with, have high titers, and can be delivered in aerosol formulation, and can transfect non-dividing cells. Pox viral vectors are large and have several sites for

10   inserting genes, they are thermostable and can be stored at room temperature. A preferred embodiment is a viral vector which has been engineered so as to suppress the immune response of the host organism, elicited by the viral antigens. Preferred vectors of this type will carry coding regions for Interleukin 8 or 10.

15   Viral vectors have higher transaction (ability to introduce genes) abilities than do most chemical or physical methods to introduce genes into cells. Typically, viral vectors contain, nonstructural early genes, structural late genes, an RNA polymerase III transcript, inverted terminal repeats necessary for replication and encapsidation, and promoters to control the

20   transcription and replication of the viral genome. When engineered as vectors, viruses typically have one or more of the early genes removed and a gene or gene/promotor cassette is inserted into the viral genome in place of the removed viral DNA. Constructs of this type can carry up to about 8 kb of foreign genetic material. The necessary functions of the removed early

25   genes are typically supplied by cell lines which have been engineered to express the gene products of the early genes in trans.

1.   Retroviral Vectors.

A retrovirus is an animal virus belonging to the virus family of Retroviridae, including any types, subfamilies, genus, or tropisms.

30   Retroviral vectors, in general, are described by Verma, I.M., Retroviral vectors for gene transfer. In MICROBIOLOGY-1985, American Society for Microbiology, pp. 229-232, Washington, (1985). Examples of methods for

using retroviral vectors for gene therapy are described in U.S. Patent Nos. 4,868,116 and 4,980,286; PCT applications WO 90/02806 and WO 89/07136; and Mulligan, (Science 260:926-932 (1993)).

5   A retrovirus is essentially a package which has packed into it nucleic acid cargo. The nucleic acid cargo carries with it a packaging signal, which ensures that the replicated daughter molecules will be efficiently packaged within the package coat. In addition to the package signal, there are a number of molecules which are needed in cis, for the replication, and packaging of the replicated virus. Typically a retroviral genome, contains

10   the gag, pol, and env genes which are involved in the making of the protein coat. It is the gag, pol, and env genes which are typically replaced by the foreign DNA that it is to be transferred to the target cell. Retrovirus vectors typically contain a packaging signal for incorporation into the package coat, a sequence which signals the start of the gag transcription unit, elements

15   necessary for reverse transcription, including a primer binding site to bind the tRNA primer of reverse transcription, terminal repeat sequences that guide the switch of RNA strands during DNA synthesis, a purine rich sequence 5' to the 3' LTR that serve as the priming site for the synthesis of the second strand of DNA synthesis, and specific sequences near the ends of

20   the LTRs that enable the insertion of the DNA state of the retrovirus to insert into the host genome. The removal of the gag, pol, and env genes allows for about 8 kb of foreign sequence to be inserted into the viral genome, become reverse transcribed , and upon replication be packaged into a new retroviral particle. This amount of nucleic acid is sufficient for the delivery of a one to

25   many genes depending on the size of each transcript. It is preferable to include either positive or negative selectable markers along with other genes in the insert.

Since the replication machinery and packaging proteins in most retroviral vectors have been removed (gag, pol, and env), the vectors are

30   typically generated by placing them into a packaging cell line. A packaging cell line is a cell line which has been transfected or transformed with a retrovirus that contains the replication and packaging machinery, but lacks

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any packaging signal. When the vector carrying the DNA of choice is transfected into these cell lines, the vector containing the gene of interest is replicated and packaged into new retroviral particles, by the machinery provided in cis by the helper cell. The genomes for the machinery are not

5   packaged because they lack the necessary signals.

2.   Adenoviral Vectors.

The construction of replication-defective adenoviruses has been described (Berkner et al., J. Virology 61:1213-1220 (1987); Massie et al., Mol. Cell. Biol. 6:2872-2883 (1986); Haj-Ahmad et al., J. Virology 57:267-

10   274 (1986); Davidson et al., J. Virology 61:1226-1239 (1987); Zhang "Generation and identification of recombinant adenovirus by liposome-mediated transfection and PCR analysis" BioTechniques 15:868-872 (1993)). The benefit of the use of these viruses as vectors is that they are limited in the extent to which they can spread to other cell types, since they

15   can replicate within an initial infected cell, but are unable to form new infectious viral particles. Recombinant adenoviruses have been shown to achieve high efficiency gene transfer after direct, *in vivo* delivery to airway epithelium, hepatocytes, vascular endothelium, CNS parenchyma and a number of other tissue sites (Morsy, J. Clin. Invest. 92:1580-1586 (1993);

20   Kirshenbaum, J. Clin. Invest. 92:381-387 (1993); Roessler, J. Clin. Invest. 92:1085-1092 (1993); Moullier, Nature Genetics 4:154-159 (1993); La Salle, Science 259:988-990 (1993); Gomez-Foix, J. Biol. Chem. 267:25129-25134 (1992); Rich, Human Gene Therapy 4:461-476 (1993); Zabner, Nature Genetics 6:75-83 (1994); Guzman, Circulation Research

25   73:1201-1207 (1993); Bout, Human Gene Therapy 5:3-10 (1994); Zabner, Cell 75:207-216 (1993); Caillaud, Eur. J. Neuroscience 5:1287-1291 (1993); and Ragot, J. Gen. Virology 74:501-507 (1993)). Recombinant adenoviruses achieve gene transduction by binding to specific cell surface receptors, after which the virus is internalized by receptor-mediated

30   endocytosis, in the same manner as wild type or replication-defective adenovirus (Chardonnet and Dales, Virology 40:462-477 (1970); Brown and Burlingham, J. Virology 12:386-396 (1973); Svensson and Persson, J.

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Virology 55:442-449 (1985); Seth, et al., J. Virol. 51:650-655 (1984); Seth, et al., Mol. Cell. Biol. 4:1528-1533 (1984); Varga et al., J. Virology 65:6061-6070 (1991); Wickham et al., Cell 73:309-319 (1993)).

A preferred viral vector is one based on an adenovirus which has had the E1 gene removed and these virons are generated in a cell line such as the human 293 cell line. In another preferred embodiment both the E1 and E3 genes are removed from the adenovirus genome.

Another type of viral vector is based on an adeno-associated virus (AAV). This defective parvovirus is a preferred vector because it can infect many cell types and is nonpathogenic to humans. AAV type vectors can transport about 4 to 5 kb and wild type AAV is known to stably insert into chromosome 19. Vectors which contain this site specific integration property are preferred. An especially preferred embodiment of this type of vector is the P4.1 C vector produced by Avigen, San Francisco, CA, which can contain the herpes simplex virus thymidine kinase gene, HSV-tk, and/or a marker gene, such as the gene encoding the green fluorescent protein, GFP.

The inserted genes in viral and retroviral usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and may contain upstream elements and response elements.

3. Expression Sequences.

Expression of the disclosed aptamers in cells also requires sequences to direct expression. It is preferred that an RNA polymerase III (pol III) promoter be used for expression. Pol III promoters generate transcripts that can be engineered to remain in the nucleus of the cell where Ku protein acts. It is preferred that a complete pol III transcription unit be used, including a pol III promoter, capping signal, and termination sequence. Pol III promoters, and other pol III transcription signals, are present in tRNA genes, 5S RNA genes, small nuclear RNA genes, and small cytoplasmic RNA

genes. Preferred pol III promoters for use in aptamer expression vectors are the human small nuclear U6 gene promoter and tRNA gene promoters. The use of U6 gene transcription signals to produce short RNA molecules *in vivo* is described by Noonberg *et al.*, *Nucleic Acids Res.* 22:2830-2836 (1995),

5   and the use of tRNA transcription signals is described by Thompson *et al.*, *Nucleic Acids Res.*, 23:2259-2268 (1995).

Many pol III promoters are internal, that is, they are within the transcription unit. Thus, these pol III transcripts include promoter sequences. To be useful for expression of aptamer molecules, these promoter sequences

10   should not interfere with the structure or function of the aptamer. The U6 gene promoter is not internal (Kunkel and Pederson, *Nucleic Acids Res.* 18:7371-7379 (1989); Kunkel *et al.*, *Proc. Natl. Acad. Sci. USA* 83:8575-8579 (1987); Reddy *et al.*, *J. Biol. Chem.* 262:75-81 (1987)). Suitable pol III promoter systems useful for expression of aptamer molecules

15   are described by Hall *et al.*, *Cell* 29:3-5 (1982), Nielsen *et al.*, *Nucleic Acids Res.* 21:3631-3636 (1993), Fowlkes and Shenk, *Cell* 22:405-413 (1980), Gupta and Reddy, *Nucleic Acids Res.* 19:2073-2075 (1990), Kickoefer *et al.*, *J. Biol. Chem.* 268:7868-7873 (1993), and Romero and Blackburn, *Cell* 67:343-353 (1991). The use of pol III promoters for expression of RNA

20   molecules is also described in WO 95/23225 by Ribozyme Pharmaceuticals, Inc.

In many contexts, it is preferred that expression of the aptamers is regulated, and in particular that the aptamers are expressed in a tissue- or cell-specific manner. For example, where a particular tissue or cell type is th

25   therapeutic focus, expression directed at that tissue or cell type is preferred. Many tissue- and cell-specific promoters are known and can be used for the expression of the disclosed aptamers. An example of this type of promoter would be the prostate specific antigen promoter, which is specifically activated in prostate cells and has been utilized for the targeting of anticancer

30   therapeutics of prostate tumors.

RNA fusion constructs can be produced which aid in the correct and specific targeting of expressed RNA molecules. These types of RNA fusion

constructs are discussed in detail in Good et al., "Expression of Small
Therapeutic RNAs in Cell Nuclei", *Gene Therapy*, 4:45-54 (1997).
Expression vectors based on human tRNA(met) and U6 snRNA promoters
are useful for targeting RNA expression and to render the resulting RNA
5   transcripts more resistant to degradation.

Other promoters useful for expressing the disclosed oligomers in
mammalian host cells may be obtained from various sources, for example,
the genomes of viruses such as: polyoma, Simian Virus 40 (SV40),
adenovirus, retroviruses, hepatitis-B virus and most preferably
10   cytomegalovirus, or from heterologous mammalian promoters, e.g. beta actin
promoter. The early and late promoters of the SV40 virus are conveniently
obtained as an SV40 restriction fragment which also contains the SV40 viral
origin of replication (Fiers et al., Nature, 273: 113 (1978)). The immediate
early promoter of the human cytomegalovirus is conveniently obtained as a
15   HindIII E restriction fragment (Greenway, P.J. et al., Gene 18: 355-360
(1982)). Of course, promoters from the host cell or related species also are
useful herein.

Enhancer generally refers to a sequence of DNA that functions at no
fixed distance from the transcription start site and can be either 5' (Laimins,
20   L. et al., Proc. Natl. Acad. Sci. 78: 993 (1981)) or 3' (Lusky, M.L., et al.,
Mol. Cell Bio. 3: 1108 (1983)) to the transcription unit. Furthermore,
enhancers can be within an intron (Banerji, J.L. et al., Cell 33: 729 (1983))
as well as within the coding sequence itself (Osborne, T.F., et al., Mol. Cell
Bio. 4: 1293 (1984)). They are usually between 10 and 300 bp in length,
25   and they function in cis. Enhancers function to increase transcription from
nearby promoters. Enhancers also often contain response elements that
mediate the regulation of transcription. Promoters can also contain response
elements that mediate the regulation of transcription. Enhancers often
determine the regulation of expression of a gene. While many enhancer
30   sequences are now known from mammalian genes (globin, elastase, albumin,
α-fetoprotein and insulin), typically one will use an enhancer from a
eukaryotic cell virus. Preferred examples are the SV40 enhancer on the late

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side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

5   The promotor and/or enhancer may be specifically activated either by light or specific chemical events which trigger their function. Systems can be regulated by reagents such as tetracycline and dexamethasone. There are also ways to enhance viral vector gene expression by exposure to irradiation, such as gamma irradiation, or alkylating chemotherapy drugs.

10   Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells) may also contain sequences necessary for the termination of transcription which may affect mRNA expression. These regions are transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding tissue factor protein. The 3' untranslated regions also include transcription termination sites. It is

15   preferred that the transcription unit also contain a polyadenylation region. One benefit of this region is that it increases the likelihood that the transcribed unit will be processed and transported like mRNA. The identification and use of polyadenylation signals in expression constructs is well established. It is preferred that homologous polyadenylation signals be

20   used in the transgene constructs. In a preferred embodiment of the transcription unit, the polyadenylation region is derived from the SV40 early polyadenylation signal and consists of about 400 bases (Figure 1). It is also preferred that the transcribed units contain other standard sequences alone or in combination with the above sequences improve expression from, or

25   stability of, the construct.

D.   Capture Tags

Isolation of Ku:oligomer complexes can be facilitated through the use of capture tags coupled to the oligomer. As used herein, a capture tag is any compound that can be associated with a synthesized RNA molecule and which

30   can be used to separate compounds or complexes having the capture tag from those that do not. Preferably, a capture tag is a compound, such as a ligand or hapten, that binds to or interacts with another compound, such as a ligand-

binding molecule or an antibody. It is also preferred that such interaction between the capture tag and the capturing component be a specific interaction, such as between a hapten and an antibody or a ligand and a ligand-binding molecule.

5   Suitable capture tags include hapten or ligand molecules that can be coupled to the 5' end of the synthesized RNA molecule. Preferred capture tags, described in the context of nucleic acid probes, have been described by Syvanen *et al.*, *Nucleic Acids Res.*, **14**:5037 (1986)). Preferred capture tags include biotin, which can be incorporated into nucleic acids (Langer *et al.*,

10   *Proc. Natl. Acad. Sci. USA* **78**:6633 (1981)) and captured using streptavadin or biotin-specific antibodies. A preferred hapten for use as a capture tag is digoxygenin (Kerkhof, *Anal. Biochem.* **205**:359-364 (1992)). Many compounds for which a specific antibody is known or for which a specific antibody can be generated can be used as capture tags. Such capture tags can

15   be captured by antibodies which recognize the compound. Antibodies useful as capture tags can be obtained commercially or produced using well established methods. For example, Johnstone and Thorpe, *Immunochemistry In Practice* (Blackwell Scientific Publications, Oxford, England, 1987), on pages 30-85, describe general methods useful for producing both polyclonal and monoclonal

20   antibodies.

           Another preferred capture tag are anti-antibody antibodies. Such anti-antibody antibodies and their use are well known. For example, anti-antibody antibodies that are specific for antibodies of a certain class (for example, IgG, IgM), or antibodies of a certain species (for example, anti-rabbit antibodies) are

25   commonly used to detect or bind other groups of antibodies. Thus, one can have an antibody to the capture tag and then this antibody:capture tag:RNA complex can then be purified by binding to an antibody for the antibody portion of the complex.

           Another preferred capture tag is one which can form selectable

30   cleavable covalent bonds with other molecules of choice. For example, a preferred capture tag of this type is one which contains a sulfer atom. An RNA molecule which is associated with this capture tag can be purified by retention

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on a thiolpropyl sepharose column. Extensive washing of the column removes unwanted molecules and reduction with β-mercaptoethanol, for example, allows the desired RNA molecules to be collected after purification under relatively gentle conditions (See Lorsch and Szostak, 1994 for a reduction to

5   practice of this type of capture tag).

One preferred type of capture tag is a nucleotide cap analog, such as a 5'-5' guanosine dinucleotide cap. The capped aptamer would be incubated with a Ku-containing biochemical fraction, and a fusion protein containing the murine cap-binding protein would then be used to recover the aptamer-

10   bound complex. This procedure has an advantage over antibody co-precipitation methods because the use of an aptamer that targets the DNA-binding site itself completely eliminates artifactual co-precipitation mediated by contaminating DNA, which is a major problem with co-precipitation of Ku protein. Bound proteins can be identified by SDS-PAGE, followed by

15   other appropriate analytical methods.

Capture tags (and the associated oligomer) are isolated by interacting the capture tag with an appropriate molecule that binds the capture tag. These molecules, herein are referred to as capture tag receptors (CTRs). These CTRs can be associated with a solid support. When capture tag complexes are bound

20   to CTRs attached to solid supports they can be effectively purified from the unwanted molecules because the solid support allows for successive washing to remove unbound molecules.

Supports that the CTRs can be coupled to can be any solid material to which the CTRs can be adhered or coupled. This includes materials such as

25   acrylamide, cellulose, nitrocellulose, glass, polystyrene, polyethylene vinyl acetate, polypropylene, polymethacrylate, polyethylene, polyethylene oxide, glass, polysilicates, polycarbonates, teflon, fluorocarbons, nylon, silicon rubber, polyanhydrides, polyglycolic acid, polylactic acid, polyorthoesters, polypropylfumerate, collagen, glycosaminoglycans, and polyamino acids.

30   Supports can have any useful form including thin films or membranes, beads, bottles, dishes, fibers, woven fibers, shaped polymers, particles and

microparticles. Preferred forms of supports are plates and beads. The most preferred form of beads are magnetic beads.

Methods for immobilization of oligonucleotides to substrates are well established. Oligonucleotides, including oligonucleotide capture docks, can be coupled to substrates using established coupling methods. For example, suitable attachment methods are described by Pease *et al., Proc. Natl. Acad. Sci. USA* **91**(11):5022-5026 (1994), and Khrapko *et al., Mol Biol (Mosk) (USSR)* 25:718-730 (1991). A method for immobilization of 3'-amine oligonucleotides on casein-coated slides is described by Stimpson *et al., Proc. Natl. Acad. Sci. USA* **92**:6379-6383 (1995). A preferred method of attaching oligonucleotides to solid-state substrates is described by Guo *et al., Nucleic Acids Res.* 22:5456-5465 (1994).

Methods for immobilizing antibodies to solid-state substrates are well established. Immobilization can be accomplished by attachment, for example, to aminated surfaces, carboxylated surfaces or hydroxylated surfaces using standard immobilization chemistries. Examples of attachment agents are cyanogen bromide, succinimide, aldehydes, tosyl chloride, avidin-biotin, photocrosslinkable agents, epoxides and maleimides. A preferred attachment agent is glutaraldehyde. These and other attachment agents, as well as methods for their use in attachment, are described in *Protein immobilization: fundamentals and applications*, Richard F. Taylor, ed. (M. Dekker, New York, 1991), Johnstone and Thorpe, *Immunochemistry In Practice* (Blackwell Scientific Publications, Oxford, England, 1987) pages 209-216 and 241-242, and *Immobilized Affinity Ligands*, Craig T. Hermanson *et al.*, eds. (Academic Press, New York, 1992). Antibodies can be attached to a support by chemically cross-linking a free amino group on the antibody to reactive side groups present within the solid-state support. For example, antibodies may be chemically cross-linked to a support that contains free amino or carboxyl groups using glutaraldehyde or carbodiimides as cross-linker agents. In this method, aqueous solutions containing free antibodies are incubated with the solid-state substrate in the presence of glutaraldehyde or carbodiimide. For crosslinking with glutaraldehyde the reactants can be incubated with 2%

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glutaraldehyde by volume in a buffered solution such as 0.1 M sodium cacodylate at pH 7.4. Other standard immobilization chemistries are known by those of skill in the art.

5   In addition, non-antibody proteins such as streptavidin, can be linked using similar methods. Many protein and antibody columns are commercially available as well as specifically derivatized supports for conjugation to the CTRs.

E.   Kits

The disclosed oligomers can also be used in a reagent kit. For
10   example, kits for identification of the Ku protein or proteins that bind the Ku protein which contains, for example, an oligomer and suitable buffer substances. In this case the oligomer and the buffer substances can be present in the form of solutions, suspensions or solids such as powders or lyophilisates. The reagents can be present together, separated from one
15   another or optionally also on a suitable carrier. The disclosed oligomers can also be used as a diagnostic agent or to identify the function of unknown genes.

II.   Methods

The disclosed oligomers can be used for any purpose involving Ku
20   binding to DNA, including methods to affect Ku activities and processes that depend on Ku activity and isolation of proteins that interact with Ku. For example, the disclosed oligomers can be used in pharmaceutical mixtures that contain one or several oligomers as the active substance, and, optionally, pharmaceutically acceptable auxiliary substances, additives and carriers.
25   Such pharmaceutical oligomers are suitable for the production of an agent to specifically inhibit Ku DNA binding activity in cells or inhibit the activity of DNA-PK.

A.   Method To Sensitize Cells To Agents

By introducing anti-Ku aptamers into cells, they can sensitize cells
30   and tissues to the cytotoxic effects of therapeutic radiation and DNA-damaging drugs. This can be useful, for example, in cancer therapy where cancer cells can be made more sensitive to chemotherapeutic agents. Ku

protein and DNA-PK are important in the repair of radiation-induced DNA damage. If damage cannot be repaired, cells die. The cytotoxic effect of ionizing radiation forms the basis for radiation therapy, which is widely used in the treatment of human cancer. The efficacy of radiation therapy is

5   currently limited by the radiation resistance of certain tumors (for example, glioblastomas) and by the side effects caused by irradiation of nearby normal tissues (for example, in treatment of breast and cervical cancer). Therefore, it is expected to be useful to have a means for sensitizing target cells and tissues to therapeutic radiation.

10   Inhibition of Ku functions can leave cells more sensitive to a variety of agents such as agents that damage DNA (discussed above) and agents that interfere with DNA topoisomerase function. For example, etoposides prevent cell division by interfering with DNA topoisomerase function. Jin et al., "Differential Etoposide Sensitivity of Cells Deficient in the Ku and

15   DNA-PKcs Components of the DNA-dependent Protein Kinase" *Carcinogenesis* 19:965-97 (1998), describe the increased sensitivity of cells with Ku mutations to etoposides, which are inhibitors of DNA Topoisomerase II. Thus, a preferred therapeutic use of the disclosed oligomers is in combination with agents that render cells more sensitive to

20   reduced Ku function. In this context, the aptamers can increase the effectiveness of the agent.

       B.   Identification Of Cellular RNA Sequences Predicted That Bind Ku Protein.

       The disclosed aptamers are useful for identifying naturally occurring

25   RNAs that bind to Ku protein. The existence of RNAs that bind tightly to Ku protein is consistent with suggestions that Ku protein function may be regulated to some extent by RNA in the normal cell. (Reeves, J. Exp. Med. 161, 18-39 (1985)). One site where such interactions might occur is in the nucleolus. The association of Ku protein with the nucleolus has been shown

30   to be RNase sensitive (Reeves, J. Exp. Med. 161, 18-39 (1985)). A number of studies have shown that DNA-PK and Ku protein have an ability to regulate RNA polymerase I, which is localized in the nucleolus (Hoff et al.,

Proc. Natl. Acad. Sci. USA 91, 762-766 (1994); Kuhn et al., Genes and
Development 9, 193-203 (1995); Labhart, Proc. Natl. Acad. Sci. USA 92,
2934-2938 (1995)). If, however, Ku protein associates with nucleolar RNAs,
this would provide a potential targeting mechanism. Nucleoli contain a

5   multitude of discrete small nucleolar RNAs, as well as nascent ribosomal
RNA (reviewed in Smith & Steitz, Cell 89, 669-672 (1997)). The consensus
sequences present in the aptamers can be used to predict whether particular
nucleolar RNAs or other cellular RNAs are likely to bind to Ku protein.
Cellular RNA sequences that become available in public databases can be

10   searched to determine whether there is identity with the consensus sequences
identified in the aptamers. Therefore, the disclosed aptamers are useful tools
for predicting whether naturally occurring RNAs are likely to bind to Ku
protein.

C.   Identification Of Proteins That Interact With Ku Protein.

15   The disclosed aptamers can also be used to identify proteins that
interact with Ku protein. This can be accomplished, for example, by
attaching a chemical capture tag to the aptamer which allows the aptamer to
be captured on a solid matrix. Ku aptamers attached to a chemical capture
tag can be isolated on, for example, a solid support. The aptamer can then be

20   used to isolate Ku protein from a mixture. Any other molecules or proteins
attached to the Ku protein can then be isolated also.

        Another method to detect proteins that interact with Ku protein is to
form complexes between radiolabeled aptamer and Ku protein, further
incubate these complexes with the protein or mixture to be tested, and

25   analyze the complexes by native polyacrylamide gel electrophoresis. The
aptamer-Ku protein complexes migrate as a discrete species at a defined
position. The mobility of this species will be shifted and it will be found at a
different position on the gel when additional proteins are bound.

        The same assay can be adapted to detect proteins that promote

30   association of Ku protein and DNA-PKcs. Because the aptamers do not
activate DNA-PK enzyme activity, it is expected that they will not, in
themselves, promote assembly of Ku protein and DNA-PKcs into a

functional DNA-PK complex. That is, when purified DNA-PKcs are incubated with aptamer-Ku protein complexes, there should be no shift in mobility. This property can serve as an assay for proteins that promote association of Ku protein and DNA-PKcs. Complexes will be formed

5   between radiolabeled aptamer and Ku protein, and will be further incubated with DNA-PKcs and the protein or mixture to be tested. Complexes will be analyzed by native polyacrylamide gel electrophoresis. The mobility of the aptamer-Ku protein complex will be shifted when DNA-PKcs and additional proteins are bound. The presence of DNA-PKcs in the complex will be

10   ascertained by determining whether monoclonal antibodies against DNA-PKcs further shift the mobility.

Although a number of antibodies to Ku protein have been described, aptamers have different and complementary properties. A variety of commercially available monoclonal antibodies (N3H10, N9C1, 111, 162,

15   and S5C11) have been screened, as described in the examples, and all were found to produce supershifts in an electrophoretic mobility shift assay, indicating that they recognize epitopes outside the DNA binding site. By contrast, the aptamers competitively inhibited DNA binding, indicating that they are targeted directly to the DNA binding site of Ku protein. This

20   property makes the aptamers uniquely useful for probing the role of Ku protein in various physiological processes.

D.   Manipulation Of Ku Activity In Cells.

The disclosed aptamers are also useful as tools for manipulation of Ku activity in cells. For this purpose, the aptamers should be expressed in or

25   delivered to cells. There are a number of techniques that can be used for targeted expression of small RNAs in the nuclear compartment (Sullenger et al., Mol. Cell. Biol. 10, 6512-6523 (1990); Thompson et al., Nucleic Acids Res. 23, 2259-2268 (1995); Good et al., Gene Therapy 4:45-54 (1997)). Introduction of aptamers to cells will be useful for investigations of the

30   physiological role of DNA-PK and other molecules and processes that Ku affects. Although DNA-PK is clearly involved in DNA repair, it has also been proposed that it is involved in a number of other processes, including

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the stress response, viral infection, and transcriptional regulation (McConnell & Dynan, Curr. Opin. Cell Biol. 8, 325-330 (1996) and references therein). A mechanism for manipulating DNA-PK activity *in vivo* is expected to provide a path for investigating the role of Ku protein in various

5   physiological processes.

E.   Use Of Aptamers To Treat Autoimmune Disease.

The disclosed aptamers can also be used to treat autoimmune disease in patients producing anti-Ku antibodies. Antibodies to Ku protein occur in 15-20% of patients with systemic lupus erythematosus, scleroderma,

10   myositis, and Sjogren's syndrome (Yaneva & Arentt, Clinical & Experimental Immunology 76, 366-372 (1989)). The immunogen in such cases may be a Ku protein-DNA complex, such as might be released from damaged or dying tissue. The disclosed aptamers can be used to disrupt such complexes, thus mitigating this chronic stimulus to the immune system.

15   For example, an aptamer may bind to Ku protein in a manner that does not allow an antibody to bind to Ku protein or may inhibit Ku protein binding to surface immunoglobulins on B cells. It has been suggested that self antigens are captured by antigen-specific receptors (surface immunoglobulins) on autoreactive B lymphocytes. (Craft and Fatenejad,

20   *Arthritis and Rheumatism*, 40:1374-1382 (1997)). Thus, an aptamer that interferes with the binding of the Ku protein to the B cell, or which alters subsequent processing, is expected to block the chain of events leading to autoimmune disease.

F.   Delivery Of Oligomers To Cells.

25   A variety of methods are available for delivering the disclosed oligomers to cells. For example, in general, the disclosed oligomers can be incorporated within or on microparticles or liposomes. Microparticles or liposomes containing the disclosed oligomers can be administered systemically, for example, by intravenous or intraperitoneal administration,

30   in an amount effective for delivery of the disclosed oligomers to targeted cells. Other possible routes include trans-dermal or oral, when used in conjunction with appropriate microparticles. Generally, the total amount of

the liposome-associated oligomer administered to an individual will be less than the amount of the unassociated oligomer that must be administered for the same desired or intended effect.

5   Oligomers including various polymers such as the polylactic acid and polyglycolic acid copolymers, polyethylene, and polyorthoesters and the disclosed oligomers can be delivered locally to the appropriate cells by using a catheter or syringe. Other means of delivering such oligomers locally to cells include using infusion pumps (for example, from Alza Corporation, Palo Alto, California) or incorporating the oligomers into polymeric implants

10   (see, for example, Johnson and Lloyd-Jones, eds., *Drug Delivery Systems* (Chichester, England: Ellis Horwood Ltd., 1987), which can effect a sustained release of the therapeutic oligomers to the immediate area of the implant.

       Delivery of the disclosed oligomers can be facilitated by targeting

15   them to particular tissue or cell types. For example, the disclosed oligomers may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989);

20   Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). Vehicles such as "stealth" and other antibody

25   conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor

30   tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either

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constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes.

5   The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration,

10   type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis has been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

The oligomers can also be delivered to cells and expressed within the targeted cells using gene transfer technology based on viral and retroviral

15   vectors. Gene transfer can be obtained using direct transfer of genetic material, in a plasmid or viral vector, or via transfer of genetic material. Further, these methods can be used to target certain diseases and cell populations by using the targeting characteristics of the carrier. Transfer vectors can be any nucleotide construction used to deliver genes into cells

20   (e.g., a plasmid), or as part of a general strategy to deliver genes, e.g., as part of recombinant retrovirus or adenovirus (Ram et al. Cancer Res. 53:83-88, (1993)). Appropriate means for transfection, including viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example,

25   Wolff, J. A., et al., Science, 247, 1465-1468, (1990); and Wolff, J. A. Nature, 352, 815-818, (1991).

III.   Definitions

As used herein, the term "aptamer" is an oligomer or oligonucleotide that folds into a specific conformation and binds a target biomolecule.

30   As used herein, oligomer refers to oligomeric molecules composed of subunits where the subunits can be of the same class (such as nucleotides) or a mixture of classes (such as nucleotides and ethylene glycol). It is preferred

that the disclosed oligomers be oligomeric sequences, non-nucleotide linkers, or a combination of oligomeric sequences and non-nucleotide linkers. It is more preferred that the disclosed oligomers be oligomeric sequences. Oligomeric sequences are oligomeric molecules where each of the subunits

5 includes a nucleobase (that is, the base portion of a nucleotide or nucleotide analogue) which can interact with other oligomeric sequences in a base-specific manner. The hybridization of nucleic acid strands is a preferred example of such base-specific interactions. Oligomeric sequences preferably are comprised of nucleotides, nucleotide analogues, or both, or are

10 oligonucleotide analogues.

Non-nucleotide linkers can be any molecule, which is not an oligomeric sequence, that can be covalently coupled to an oligomeric sequence. Preferred non-nucleotide linkers are oligomeric molecules formed of non-nucleotide subunits. Examples of such non-nucleotide linkers are

15 described by Letsinger and Wu, (*J. Am. Chem. Soc.* 117:7323-7328 (1995)), Benseler *et al.*, (*J. Am. Chem. Soc.* 115:8483-8484 (1993)) and Fu *et al.*, (*J. Am. Chem. Soc.* 116:4591-4598 (1994)). Preferred non-nucleotide linkers, or subunits for non-nucleotide linkers, include substituted or unsubstituted C_1-C_{10} straight chain or branched alkyl, substituted or unsubstituted C_2-C_{10}

20 straight chain or branched alkenyl, substituted or unsubstituted C_2-C_{10} straight chain or branched alkynyl, substituted or unsubstituted C_1-C_{10} straight chain or branched alkoxy, substituted or unsubstituted C_2-C_{10} straight chain or branched alkenyloxy, and substituted or unsubstituted C_2-C_{10} straight chain or branched alkynyloxy. The substituents for these

25 preferred non-nucleotide linkers (or subunits) can be halogen, cyano, amino, carboxy, ester, ether, carboxamide, hydroxy, or mercapto.

As used herein, nucleoside refers to adenosine, guanosine, cytidine, uridine, 2'-deoxyadenosine, 2'-deoxyguanosine, 2'-deoxycytidine, or thymidine. A nucleoside analogue is a chemically modified form of

30 nucleoside containing a chemical modification at any position on the base or sugar portion of the nucleoside. As used herein, the term nucleoside analogue encompasses, for example, both nucleoside analogues based on

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naturally occurring modified nucleosides, such as inosine and pseudouridine, and nucleoside analogues having other modifications, such as modifications to the 2' position of the sugar. As used herein, nucleotide refers to a phosphate derivative of nucleosides as described above, and a nucleotide

5   analogue is a phosphate derivative of nucleoside analogues as described above. The subunits of oligonucleotide analogues, such as peptide nucleic acids, are also considered to be nucleotide analogues.

As used herein, a ribonucleotide is a nucleotide having a 2' hydroxyl function. Analogously, a 2'-deoxyribonucleotide is a nucleotide having only

10   2' hydrogens. Thus, ribonucleotides and deoxyribonucleotides as used herein refer to naturally occurring nucleotides having nucleoside components adenosine, guanosine, cytidine, and uridine, or 2'-deoxyadenosine, 2'-deoxyguanosine, 2'-deoxycytidine, and thymidine, respectively, without any chemical modification. Ribonucleosides, deoxyribonucleosides,

15   ribonucleoside analogues and deoxyribonucleoside analogues are similarly defined except that they lack the phosphate group, or an analogue of the phosphate group, found in nucleotides and nucleotide analogues.

As used herein, oligonucleotide analogues are polymers of nucleic acid-like material with nucleic acid-like properties, such as sequence

20   dependent hybridization, that contain at one or more positions, a modification away from a standard RNA or DNA nucleotide. A preferred example of an oligonucleotide analogue is peptide nucleic acid.

As used herein, base pair refers to a pair of nucleotides or nucleotide analogues which interact through one or more hydrogen bonds. The term

25   base pair is not limited to interactions generally characterized as Watson-Crick base pairs, but includes non-canonical or sheared base pair interactions (Topal and Fresco, *Nature* 263:285 (1976); Lomant and Fresco, *Prog. Nucl. Acid Res. Mol. Biol.* 15:185 (1975)).

As used herein the term "gene" when used in the context of genetic

30   vectors refers to the genetic material of interest that is to be transferred to the host organism. For example, the "gene" to be transferred could be a

sequence which codes for an aptamer and when transcribed would produce a functional aptamer.

The present invention will be further understood by reference to the following examples.

5   **Examples**

Example 1:   Preparing the RNA Pool and Screening for Ku protein binding.

Because there was little previous information about Ku protein-RNA interactions, the RNA binding properties of Ku protein were systematically

10   investigated using SELEX (systematic evolution of ligands by exponential enrichment) technology. With this technology, it is possible to identify oligonucleotides from a large, random pool that bind to a ligand of interest. The SELEX method has been used to identify oligonucleotides that bind to nucleic acid binding proteins, to non-nucleic acid binding proteins, and to

15   small molecules (reviewed in Gold et al., Annu. Rev. Biochem. 64, 763-797 (1995)).

The RNA pool was prepared by synthesis of single stranded RNA from a template comprising a region of conserved sequences and a region of randomized and/or biased sequences.

20   Synthesis of the RNA Pool

SELEX protocols and the template used to synthesize the nonselected RNA pool were obtained from Dr. Hang Chen and Dr. Larry Gold (University of Colorado) and modified as described below. The major change to the SELEX protocol was to increase the KCl concentration during

25   sequential rounds of SELEX to maintain stringency. Protocols can also be obtained from Tuerk & Gold, Science 249, 505-510 (1990), and U.S. Patent Numbers 5,270,163 and 5,567,588. HeLa cell nuclear extracts and oligonucleotides were obtained from Dr. Mark Anderson and Ms. Kirsten Strickler. Chemicals were obtained from Sigma, Fisher Scientific, antibodies

30   from Neomarkers, nitrocellulose filters from Millipore, cloning vectors from Promega and Invitrogen, translation kits from Ambion, and RNasin from Promega.

SELEX procedures to select Ku binding RNAs were carried out largely as described in Chen & Gold, Biochemistry 33, 8746-8756 (1994). The template for synthesis of the starting RNA pool was based on a DNA oligonucleotide, 5'-CCCGGATCCTAGTTCACGATGCTGCAA-(N)$_{40}$-

5   TTACGGTCTGAGAAAATATCCTCCC-3', where N indicates an equimolar mixture of A, G, C and T. This sequence was used because it is known to function well in SELEX. The 5' and 3' constant regions do not bind to Ku protein. DNA templates were generated by PCR amplification of this oligonucleotide using primer 1, 5'-CCCAAGCTTAATAC

10   GACTCACTATAGGGAGGATATTTTC TCAGACCGTAA-3', and primer 2, 5'-CCCGGATCCTAGTTCACGATGC TGCAA-3'. Amplification was carried out with 0.5 μM of the oligonucleotide containing the random segment, 2 μM primer 1 and primer 2, 1 mM dATP, dCTP, dGTP, and dTTP, 50 U/ml Taq DNA polymerase, 50 mM KCl, 10 mM Tris-HCl, pH

15   8.4, 7.5 mM MgCl$_2$, and 50 μg/mL BSA in a volume of 0.5 ml. After four cycles of amplification (93° C for 30 s, 57° C for 20 s, 73° C for 90 s), the reaction was extracted with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1 v/v/v) (PCIA), and nucleic acids were precipitated from the supernatant by addition of 0.1 vol of 3 M NaOAc pH 5.2 and 2.5 vol ethanol.

20   This template preparation was incubated for 2-3 h at 37° C in a reaction mixture containing 475 U/ml T7 RNA polymerase, 40 mM Tris-HCl, pH 8.0, 12 mM MgCl$_2$, 5 mM DTT, 1 mM spermidine, 0.002 % Triton X-100, 4 % PEG 8000, 2 mM ATP, UTP, and GTP, and 0.2 mM [a-^{32}P] CTP (1 Ci/mmol) in a final volume of 200 μl. DNase I was added (25 U/ml) and

25   incubation was continued for 30 minute. The resulting RNAs were fractionated by 10% urea-PAGE and gel slices containing full-length 92 nucleotide RNA were excised, crushed, mixed with 0.5 ml TE, 0.5 ml phenol, and 10 μl 10% sodium dodecyl sulfate, and tumbled overnight at 4° C to extract RNA. The supernatant was collected and RNA was precipitated

30   with NaOAc and EtOH as described above. RNA was dissolved in TE, denatured at 100° C for 2 minute, and renatured by adjusting to 5 mM

MgCl₂, cooling rapidly to 0° C, and incubating for 30 minute. The amount of
RNA recovered was estimated by liquid scintillation counting.

Protein purification

Native DNA-PKcs and Ku protein were purified from HeLa cell

5   nuclear extracts as previously described in Dvir et al., J. Biol. Chem
268(14):10440-10447 (1993), except that the Phenyl-Superose and Mono S
steps were omitted. Monoclonal antibodies to Ku protein were obtained from
Neomarkers (Fremont, CA).

Selection of Ku-binding RNAs

10   To perform *in vitro* selections, the RNA pool was mixed with
purified Ku protein under conditions for complex binding. RNA and Ku
protein were incubated for 30 minute at room temperature in a buffer
containing 25 mM Tris-HCl, pH 7.9, 0.5 mM EDTA, 10 % glycerol, 5 mM
MgCl₂, 0.5 mM DTT, and 0.01 % Tween 20 in a final volume of 50-100 µl.

15   Concentrations of RNA, Ku protein, and KCl were variable, as specified in
Table 2. Ku protein-RNA complexes were isolated by electrophoresis on a 5
% non-denaturing polyacrylamide gel containing 25 mM Tris-HCl, pH 8.3,
190 mM glycine, and 1 mM EDTA at 300 V for one hour. In one
experiment, complexes were isolated by passage over a nitrocellulose filter

20   (Millipore 25-µm HAWP), which was washed twice with 5 ml of 25 mM
Tris-HCl, pH 7.9. RNA was eluted from the polyacrylamide gel or the filter
by tumbling in a mixture of TE, phenol, and SDS as described above.

The RNAs having a high affinity for the Ku protein relative to the
mixture were partitioned by nitrocellulose filter binding and electrophoretic

25   mobility shift assay. Another way to separate complexes is by
immunoprecipitation.

Once the enriched RNA molecules were separated from the isolated
complexes, they were amplified by reverse transcription followed by
polymerase chain reaction. The complexity of a selected pool can be

30   characterized by direct sequencing or restriction enzyme analysis.

Additional Rounds of SELEX Selection and Amplification

When additional rounds of selection were performed, purified Ku
protein (from HeLa cell nuclear extracts) was mixed with the RNA pool and
allowed to bind. Ku-RNA complexes were isolated using either a

5   nitrocellulose filter binding or an electrophoretic mobility shift assay
(EMSA). RNA that was bound to Ku protein was isolated, amplified by RT-
PCR, and again enriched based on the ability to bind to Ku protein. The
selected RNA was reverse-transcribed by incubating in a reaction mixture
containing 50 mM Tris-HCl, pH 8.3, 60 mM NaCl, 6 mM Mg(OAc)2, 10

10   mM DTT, 0.4 mM dATP, dCTP, dGTP, and dTTP, and 300 U/ml of AMV
reverse transcriptase, in a final volume of 50 µl. The resulting cDNA was
amplified by 15 cycles of PCR using primers 1 and 2 at 93°C for 30 seconds,
58°C for 20 seconds, 72°C for 90 seconds. This process was repeated 6-7
times. Selection conditions used for each round of selection are given in

15   Table 2. To maintain the stringency of selection, the RNA:protein ratio, the
KCl concentration, or both were progressively increased as shown in Table
2. Two independent selections were conducted using different batches of
starting RNA.

After six or seven rounds of selection, cDNA was synthesized from

20   the final RNA pool and amplified by 5 cycles of PCR using primer 1 and 2.
The product was digested with Bam HI and Hind III and subcloned into the
Bam HI and Hind III sites of pGEM3zf(+) (Promega) or pZero2.1
(Invitrogen). Inserts were sequenced with an m13 reverse primer using an
ABI automated sequencer.

Table 2: Summary of the Selection Parameters

		Selection I		
SELEX Round	Input RNA (nM)	Ku protein[a] (nM)	KCl[b] (mM)	Selection[c] Method
1	1305	64.1	50	NCFA
2	612.5	24.3	50	EMSA
3	860	42.6	120	EMSA
4	1130	42	120	EMSA
5	2320	64	150	EMSA
6	142	3.2	160	EMSA

		Selection II		
SELEX Round	Input RNA (nM)	Ku protein (nM)	KCl (mM)	Selection Method
1	1330	53	120	EMSA
2	1660	42	120	EMSA
3	456	14.9	150	EMSA
4	144	3.84	160	EMSA
5	1065	21.3	160	EMSA
6	2710	18.6	160	EMSA
7	436	2.4	160	EMSA

[a] The ratio of Ku protein to input RNA was gradually decreased in order to increase the stringency. [b] Salt concentration was increased to maintain high stringency. [c] Nitrocellulose filter binding assay (NCFA) or electrophoretic mobility shift assay (EMSA) were employed for selection methods.

5   RNAs from the last round of selection were cloned and sequenced using standard techniques. Table 1 presents the sequence of nineteen aptamer RNAs that were isolated that bind avidly to Ku protein. Many of the RNAs contain common sequence motifs. A number of the RNAs bind competitively with DNA to Ku protein and thereby inhibit DNA and PK

10   enzymatic activity. The ability of Ku protein to bind tightly to specific RNAs is consistent with a role for RNA in the regulation of Ku protein activity or intranuclear localization. Additionally, the identification of diverse RNAs that bind avidly to Ku protein raises the possibility that natural RNAs modulate the activity of DNA-PK *in vivo*.

15   Binding of Ku Protein

         Binding of RNA and DNA to Ku protein was measured using EMSA as shown by the representative Ku protein-RNA binding curves after successive rounds of SELEX in Figure 1. Binding curves were obtained with EMSA followed by a nonlinear least squares fit of the data. The RNA

20   pools in Figure 1 were transcribed from templates prior to the first round (nonselected), and after the fourth (4th), and the sixth (6th) rounds of SELEX using T7 RNA polymerase as described in example 2. A twenty-one base pair double-stranded DNA was end-labeled with T4 polynucleotide kinase. The DNA was arbitrarily chosen as a transcription factor binding site from

25   the human T-cell leukemia virus proviral promoter, 5'-CTCAGGCGTTGACGACAACCC-3'.

         Ku protein bound to nonselected RNA with an apparent average K_d of 24 nM. This binding was somewhat stronger than expected, given that previous work had found that Ku protein had little ability to interact with

30   tRNA or synthetic RNA polymers (Mimori & Hardin, J. Biol. Chem. 261, 10375-10379 (1986)). Pooled RNA tested after the 4th and 6th round of

SELEX showed an increased ability to bind to Ku protein. RNA obtained after the 6th round of selection bound with an apparent K_d of about 0.3 nM, which was comparable to the binding seen with a double-stranded DNA oligonucleotide under similar conditions. Although the average affinity of

5   the selected RNA was only 75-fold greater than for the nonselected pool, subsequent experiments revealed clear functional differences, as only the selected RNAs were able to inhibit biochemical activities of Ku protein.

Example 2:   Comparison of binding of RNAs to determine specificity.

Synthesis of aptamer RNAs

10   Individual aptamer RNAs were synthesized by T7 RNA polymerase using linearized plasmid template. Both radiolabeled RNA and nonradiolabeled RNA were prepared using a MEGA shortscript T7 kit (Ambion) with 8 μg linearized plasmid template. The RNA was gel-purified, heated, and refolded as described in example 1, and the final

15   concentration was determined spectrophotometrically. Secondary structures were predicted using the method of Zuker (Jaeger et al., Proc. Natl. Acad. Sci. USA 86, 7706-7710 (1989); Jaeger et al., Meth. Enzymol. 183, 281-306 (1990)) as implemented on the mfold server www.ibc.wustl.edu/%7Ezuker/rna/form1.cgi).

20   Synthesis of RNA containing the TAR sequence

Pools of RNA at various stages of selection were also characterized directly for their ability to bind to Ku protein in an electrophoretic mobility shift assay. For comparison of the aptamer RNAs binding affinity with other known RNAs, these assays also included a HIV TAR RNA, which was

25   synthesized by T7 RNA polymerase as described in the preceding section. This RNA consisted of the following HIV-derived sequence: GGGUCUCUCUGGUUAGACCAGAUCUGAGCCUGGGAGCUCUCUG GCUAACUAGGGAACCC.

TAR-containing RNAs have previously been reported to bind

30   selectively to Ku protein (Kaczmarski & Khan, Biochem. Biophys. Res. Commun. 196, 935-942 (1993)).

Binding of the TAR RNA was measurable, but weak, under the conditions of the experiments. These results confirm that TAR-containing RNA has some ability to bind to Ku protein, but indicate that the binding is not particularly strong, relative to other short RNAs. An *in vitro* yeast tRNA

5   transcript also bound very weakly to Ku protein.

Example 3:   Cloning, sequence analysis of aptamers, and K_d of aptamers.

After six and seven rounds of SELEX (Selection I and II in Table 2, respectively), the pooled RNA was reverse-transcribed, PCR-amplified, and

10   cloned into plasmid vectors. 82 clones were isolated, including 63 from Selection I and 19 from Selection II. Allowing for duplicates, the 82 clones represented 19 independent sequences, which are given in Table 1.

An equilibrium dissociation constant was determined for the binding of each of the 19 different RNAs to Ku protein. The results, given in Table

15   1, showed that most of the RNAs have a K_d of less than 2 nM.

The sequence alignment in Table 1 revealed that the RNAs can be grouped into three classes, based on the presence of conserved sequence motifs in the variable region of each aptamer. The most striking of these motifs was a sequence, GCUUUCCCANNNAC (herein designated SEQ ID

20   NO:20) (wherein N may be A, C, G or T), which was perfectly conserved in three independent RNA sequences and partially conserved in four others. A second motif, AMAUGA (herein designated SEQ ID NO:21) (wherein M may be either A or C) was perfectly conserved in three RNAs and partially conserved in seven others. A third motif, which partially overlaps the

25   second, had the sequence AACUUCGA (bases 31-38 of SEQ ID NO:15). This motif was present in one RNA and partially conserved in four others. Several RNAs fell into hybrid classes containing two of the three motifs. Subsequent analysis showed that the hybrid RNAs containing two motifs were among the most efficacious blockers of Ku protein activity in

30   functional assays.

Example 4:   Inhibition of DNA-PK activity by different RNA aptamers.

Binding of the RNA aptamers to Ku protein to determine if they are capable of regulating Ku protein activity was then measured. One of the

5   major biochemical functions of Ku protein is to regulate the activity of DNA-PK. Ku protein targets the catalytic subunit of DNA-PK (DNA-PKcs) to DNA, increasing phosphorylation activity by 5-50 fold (Dvir et al., Proc. Natl. Acad. Sci. USA 89, 11920-11924 (1992); Gottlieb & Jackson, Cell 72, 131-142 (1993)). Under some assay conditions, DNA-PK that has been

10   depleted of Ku protein can no longer be stimulated by double-stranded DNA (Dvir et al., J. Biol. Chem. 268, 10440-10447 (1993)).

Kinase assay

Peptide phosphorylation was carried out using a previously described method (Lees-Miller et al., Mol. Cell. Biol. 12, 5041-5049 (1992)) with

15   modifications. Reactions contained 1 nM Ku protein, 0.5 nM DNA-PKcs, 100 μM p53-derived peptide (EPPL$\underline{S}$QEAFADLLWKK, phosphorylation site underlined), 0.25 nM DNA fragment (308 base pair Bgl I-Blp I fragment from pHSE1 (Peterson et al., J. Biol. Chem. 270, 1449-1454 (1995b)), 4000 U/ml RNasin (Promega), 0.2 mM [g-^{32}P] ATP (1.25 Ci/mmol), 25 mM Tris-

20   HCl, pH 7.9, 25 mM MgCl$_2$, 1.5 mM DTT, 50 mM KCl, and 10% glycerol in a total volume of 20 μl. Reactions were incubated at 30° C for 45 minute, stopped by addition of 10 μl of 1 mg/ml BSA and 10 μl of 40 % TCA, and further incubated for 30 minute on ice. Precipitated protein was removed by centrifugation and 10 μl of each supernatant, which contained the

25   phosphorylated p53 peptide, was spotted on a phosphocellulose filter (Whatman P81). The filters were washed three times with 15% HOAc for 15 minutes, and incorporation of radiolabel was measured by liquid scintillation counting.

Ku-binding RNAs were able to block the ability of Ku protein to

30   activate DNA-PK. Each of the nineteen selected aptamers, as well as the nonselected RNA pool, was tested in a DNA-PK assay. Each RNA was

45

prepared by *in vitro* transcription and added to a DNA-PK phosphorylation assay.

DNA-PK assay reactions contained 0.5 nM Ku protein, 1 nM DNA-PKcs, 0.25 nM 308-base pair double-stranded DNA fragment, a synthetic

5   peptide (EPPL_S_QEAFADLLWKK) containing a DNA-PK phosphorylation site from p53 (underlined), and the indicated amounts (8, 16, 32 nM) of RNA aptamer. Native DNA-PKcs were purified from HeLa cell nuclear extracts as described in example 2. In Figure 2A, DNA-PK activity is expressed as a percentage of activity in the absence of RNA. Values shown are averages of

10   duplicate reactions with standard deviations indicated. Background phosphorylation in the absence of DNA has been subtracted.

The effect on DNA-PK activity was measured, relative to control reactions with no added RNA. Nonselected RNA had no effect on DNA-PK activity (Figure 2A). Of the selected RNAs, seven inhibited DNA-PK

15   activity by 85% or more when present at 16-32 nM (Figure 2A). Ten RNAs inhibited DNA-PK to a lesser extent and two had no effect on DNA-PK in the concentration range tested. These results indicate that at least some of the selected RNAs interact with regions of Ku protein that are critical for its biochemical activity. Since DNA-PKcs are known to have a low level of

20   activity in the absence of Ku protein (Dvir et al., J. Biol. Chem. 268, 10440-10447 (1993)), the inhibition seen with the more effective RNAs probably reflects near-total ablation of Ku protein regulatory function.

RNAs cannot activate DNA-PK directly

It was determined whether any of the RNAs were capable of

25   activating, as opposed to inhibiting, DNA-PK phosphorylation activity. None of the RNAs significantly increased DNA-PK activity in a standard assay performed in the presence of DNA. However, under these conditions, the DNA-PK was already highly active and a weak ability of RNA to activate DNA-PK might have gone undetected. Indeed, displacement of

30   DNA by a weakly activating RNA may have resulted in partial inhibition of DNA-PK activity.

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To address the question whether any of the RNAs were capable of activating DNA-PK in the absence of DNA, DNA-PK assays in the absence of DNA were performed. Kinase assays were performed as described above in the presence or absence of 0.25 nM double-stranded DNA, as indicated.

5 Reactions contained various amounts of aptamer RNA (8, 16, 32 nM).

When neither RNA nor DNA was present, DNA-PK had a low level of basal activity, as expected. The addition of double-stranded DNA increased this activity about ten-fold. Four RNAs were tested, including one that had given no effect on DNA-PK activity in the earlier assays (#52), two

10 that had given partial inhibition (SC1, SC9), and one that had given potent inhibition (SC4). None of these four RNAs showed any ability to activate DNA-PK above background levels in the absence of DNA.

Competitive binding analysis between double stranded DNA and RNA   In principle, an RNA that binds to Ku protein could inhibit DNA-PK

15 either by blocking the binding of Ku protein to DNA or by blocking the interaction between Ku protein and DNA-PKcs. To distinguish these possibilities, the RNAs were tested for their effect on Ku protein-DNA binding.

Ku protein was incubated with a radiolabeled twenty-one base pair

20 double stranded DNA (1 nM) oligonucleotide in the presence or absence of various amounts (8, 16, 32 nM) of nonradiolabeled RNA. The incubation conditions were 25 mM Tris-HCl pH 7.9, 0.5 mM EDTA, 10% glycerol, 5 mM $MgCl_2$, 0.5 mM DTT, 0.01% Tween, 120 mM KCl, 30 minute at ambient temperature. Ku protein-DNA complexes were trapped in a

25 nitrocellulose filter binding assay as described in example 1 to measure Ku-DNA complexes. The results are shown in Figure 2B, where binding is expressed as a percentage of Ku-DNA complexes detected in the absence of competitor. Values shown are averages of duplicate reactions with standard deviations indicated. Eleven cloned RNAs were tested, all of which had

30 previously been shown to inhibit DNA-PK in a phosphorylation assay. All of these RNAs competed to some extent with the double-stranded DNA oligonucleotide for binding to Ku protein. The seven RNAs that functioned

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as effective inhibitors of DNA-PK enzyme activity also proved to be
effective inhibitors of Ku protein-DNA binding activity (Figure 2B). By
contrast, RNA from the nonselected pool had no effect on Ku protein-DNA
interaction (Figure 2B). There was a rough correlation between the

5   efficiency with which individual RNAs inhibited DNA binding and DNA-
PK phosphorylation. Although not wishing to be bound by theory, the
results suggest that all of the inhibitory RNAs that were tested work
primarily by interfering with protein-DNA interactions, rather than protein-
protein interactions.

10   Both of the subunits of Ku protein have a predicted net negative
charge at neutral pH, and it may be that unfavorable electrostatic interactions
restrict interaction with aptamer RNAs over much of the protein's surface.
Given this constraint, it is perhaps not surprising that many of the aptamers
are targeted toward the DNA binding site within the Ku protein. Although

15   this site has not been fully mapped, it is likely that it contains basic residues
that are capable of electrostatic interactions with the phosphate backbone of
RNA and DNA.

To further explore the relationship between RNA and DNA binding
sites in Ku protein, Electrophoretic Mobility Shift Assays (EMSAs) were

20   performed comparing radiolabeled aptamer RNA (1 nM) and radiolabeled
twenty-one base pair double stranded DNA (1 nM) probes. Cross-
competition between RNA aptamers and DNA for Ku protein binding were
found. It was discovered that Ku protein forms stable complexes with both
types of probes, and that the complexes with RNA and DNA have similar

25   electrophoretic mobilities. Both of the RNAs that were tested, #7-3 and
#SC9, competed with DNA for binding to Ku protein. RNA #7-3 is a more
effective competitor than RNA #SC9, consistent with the results of
nitrocellulose filter binding assays. In the reciprocal experiment,
nonradiolabeled DNA fragments competed with labeled RNA for binding to

30   Ku protein. As controls, DNA and each RNA were shown to effectively
self-compete for binding to Ku protein, confirming the fidelity of the
analysis.

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Notably, neither the DNA nor the RNA competitors induced the formation of supershifted complexes. The absence of supershifted complexes provides additional evidence that RNA and DNA cannot bind simultaneously to Ku protein, but rather, bind competitively to the same site.

5   Specific inhibition of DNA-PK by aptamers in crude cell extracts

Part of the rationale for identifying RNAs that interact with Ku protein was to develop potential tools, aptamers, for *in vivo* modulation of DNA-PK activity. To be useful, such aptamers must not only recognize Ku protein, but they must be able to do so in a complex milieu containing other

10   macromolecules.

To determine whether the RNAs were specific for Ku protein, the ability of the aptamers to inhibit DNA-PK in crude HeLa cell nuclear extracts were measured. As shown in Figure 3, phosphorylation of the p53-derived peptide substrate by crude extracts is stimulated at least five-fold by

15   addition of double stranded DNA. This suggests that DNA-PK is the major kinase that phosphorylates this substrate under the conditions used. The reactions contained 2 μl HeLa cell nuclear extract (0.4 μg protein) and other components as in Figure 2. Reactions were performed in the presence of various amounts of aptamer RNA (250, 500, 1000 nM) as indicated. The

20   assays were as described above except a higher amount of DNA was used, 5.3 nM. 100 to 142.1 μM p53 was used. DNA-PK activity is expressed as a percentage of activity in the absence of RNA. Values shown are averages of duplicate reactions with standard deviations as indicated. Four different aptamer RNAs, all of which had previously been shown to inhibit purified

25   DNA-PK, were tested in the crude extract. All four reduced enzyme activity to near-basal levels. RNA from the nonselected pool had no effect at concentrations up to 1000 nM (Figure 3, lanes 5-7). These results demonstrate that the aptamers are sufficiently selective that they can recognize Ku protein and inhibit DNA-PK activity even in a crude

30   preparation.

Dissociation of Ku-DNA complexes by aptamer RNAs

Previous work has suggested that, once the Ku protein binds to double-stranded DNA, the resulting complexes are not readily disrupted by a second DNA added as competitor, except when the first and second DNAs

5   have cohesive ends (Bliss & Lane, J. Biol. Chem. 272, 5765-5773 (1997)). To find out whether aptamers were capable of capturing Ku protein from preformed complexes, an experiment was performed in which purified Ku protein (0.32 nM) was incubated with a radiolabeled double-stranded DNA (5' end labelled with T-4 polynucleotide kinase and [γ-32P] ATP) fragment

10   (0.38 nM) under conditions for forming complexes for 30 minutes at room temperature. The complexes were challenged with either aptamer RNA or RNA from the nonselected pool. Incubation was then continued and the amount of Ku-DNA complexes remaining were measured by electrophoretic mobility assay using a 5% nondenaturing polyacrylamide gel containing 25

15   mM Tris-HCl pH 8.3, 190 mM glycine, 1 mM EDTA, run for one hour at 300 V, ambient temperature.

Ku protein-DNA complexes steadily dissociated in the presence of aptamer #7-3. In contrast, the amount of Ku protein-DNA complexes remained almost unchanged when challenged with nonselected RNA. This

20   result demonstrates that the aptamer RNA can compete with double-stranded DNA for the binding of Ku protein even if the aptamer RNA is added after Ku-DNA complexes have formed.

Claims

We claim:

1. An oligomer comprising:

a sequence that specifically binds to Ku protein with an equilibrium dissociation constant less than 10 nM.

2. The oligomers according to claim 1 wherein the oligomers compete with DNA to bind Ku protein.

3. The oligomers according to claim 1 wherein the oligomer comprises at least one of the three nucleotide sequences chosen from the group comprising: GCUUUCCCANNNAC, A(A/C)AUGA, and AACUUCGA.

4. An oligomer according to claim 1 wherein the oligomer is made of RNA nucleotides.

5. An oligomer according to claim 1 wherein the oligomer comprises the components A, A', B, C, C', D, and E, wherein,

the components are arranged 5'-A-B-C-D-C'-E-A'-3' wherein,

component A hybridizes to component A',

component C hybridizes to component C',

components B and E form bulges, and

component D forms a structure which is either a bulge or a loop.

6. An oligomer according to claim 5 wherein the secondary structure formed is represented by Figure 6A.

7. An oligomer comprising the sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19.

8. A method of altering an activity of Ku protein comprising bringing into contact an oligomer of claim 1 and Ku protein.

9. The method of claim 8 wherein the Ku protein activity is the effect of Ku on DNA-PK.

10. The method of claim 8 wherein the bringing into contact is accomplished by delivering the oligomer to a cell or by expressing the oligomer in a cell.

11. The method of claim 10 wherein the cell becomes more sensitive to an agent when the oligomer is delivered to or expressed in the cell.

12. The method of claim 11 wherein the agent is a mutagen.

13. The method of claim 12 wherein the mutagen is radiation.

14. The method of claim 11 wherein the agent is a topoisomerase inhibitor.

15. A method of identifying physiological effects of Ku protein comprising exposing a cell to an oligomer of claim 1, and identifying a physiological difference between the cell exposed to the oligomer and a cell not exposed to the oligomer.

16. A method of isolating proteins that interact with Ku protein comprising

incubating an oligomer of claim 1 with a sample containing Ku protein such that oligomer-Ku complexes can form,

recovering the oligomer-Ku complexes, and

recovering any protein associated with the oligomer-Ku complexes.

17. The method of claim 16 wherein the oligomer further comprises a capture tag, and wherein the oligomer-Ku complex is recovered using the capture tag.
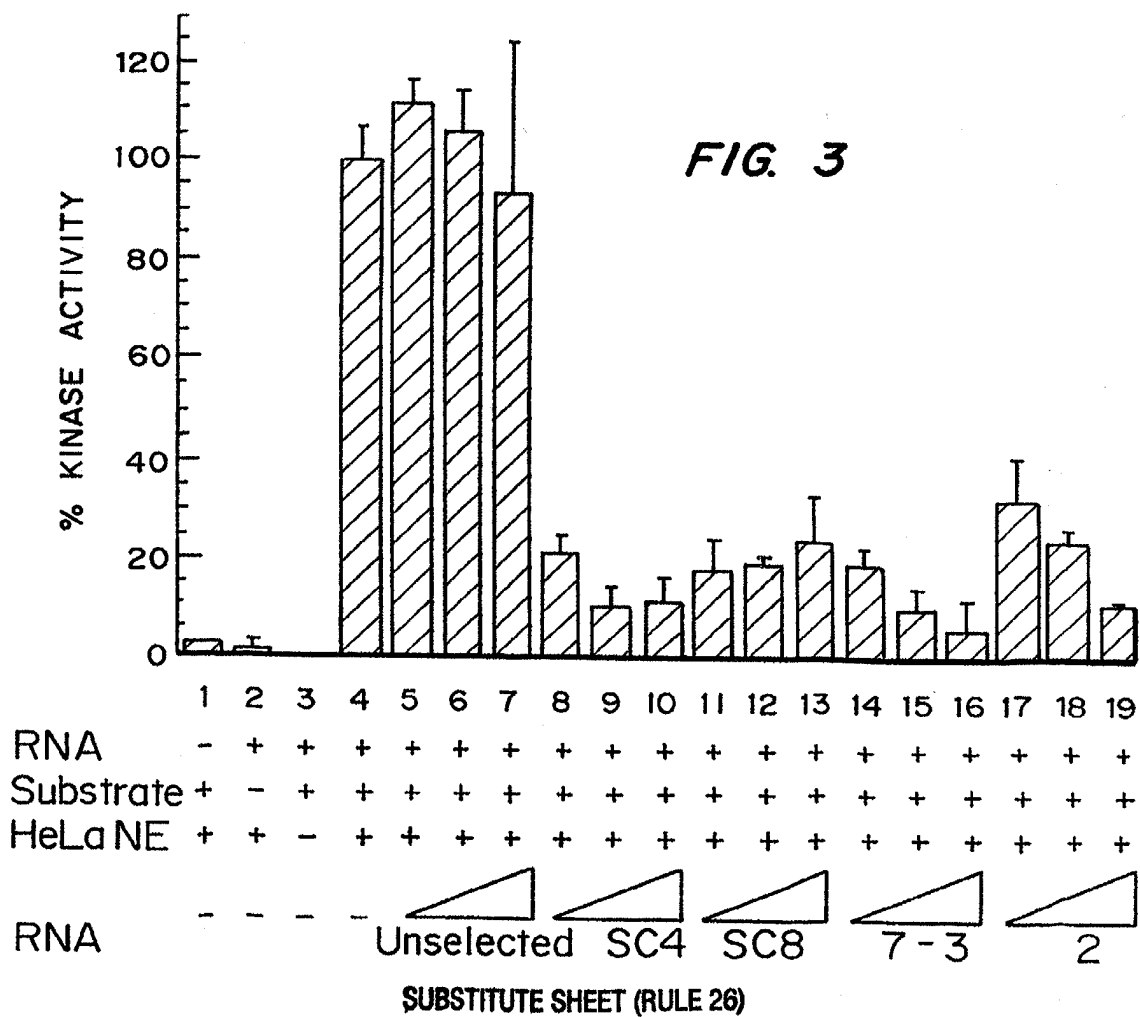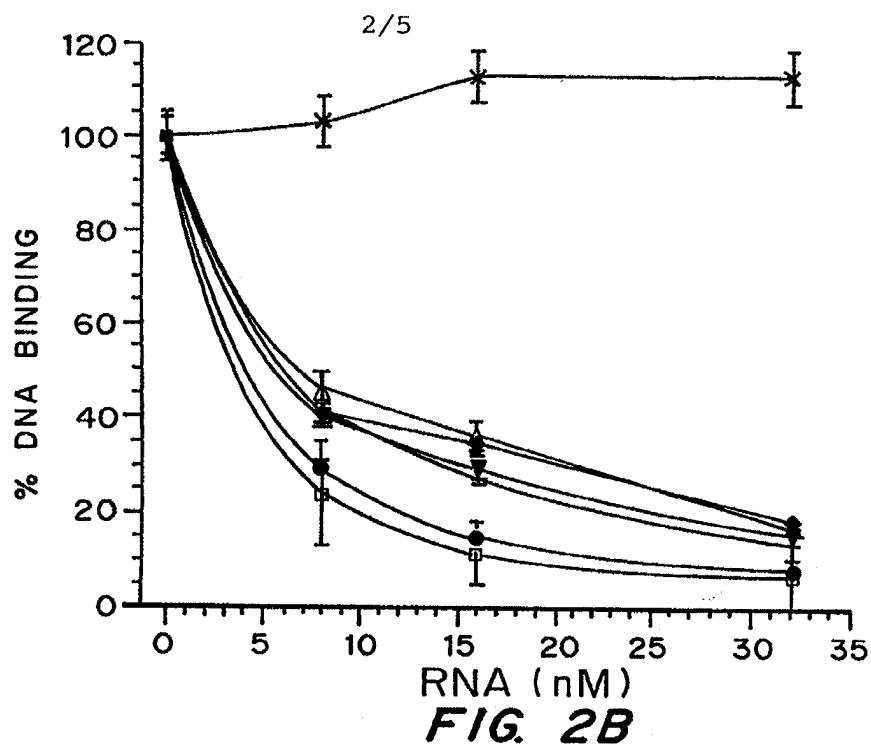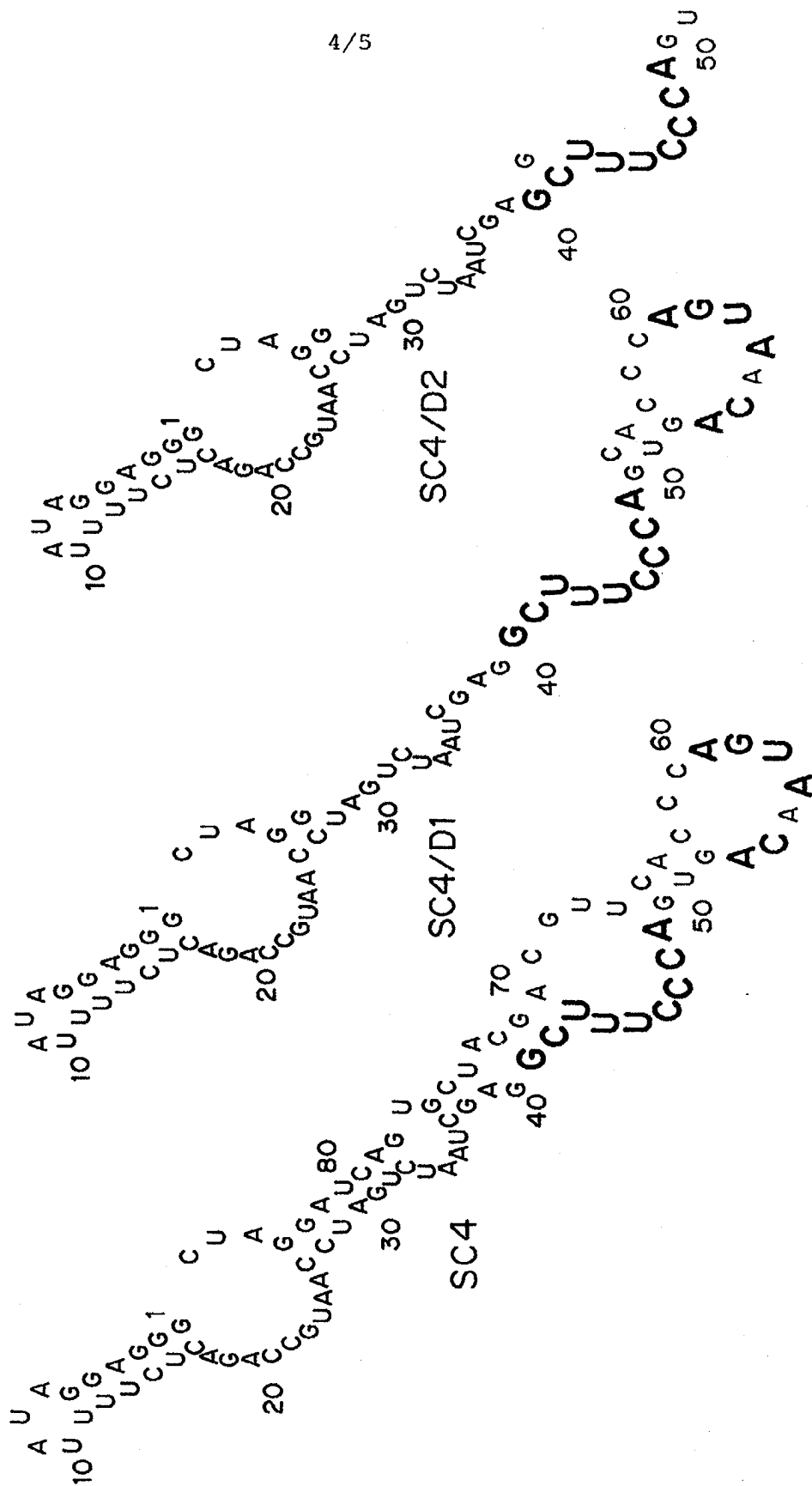
1/5

FIG. 1

FIG. 2A

FIG. 2B

FIG. 3

FIG. 4
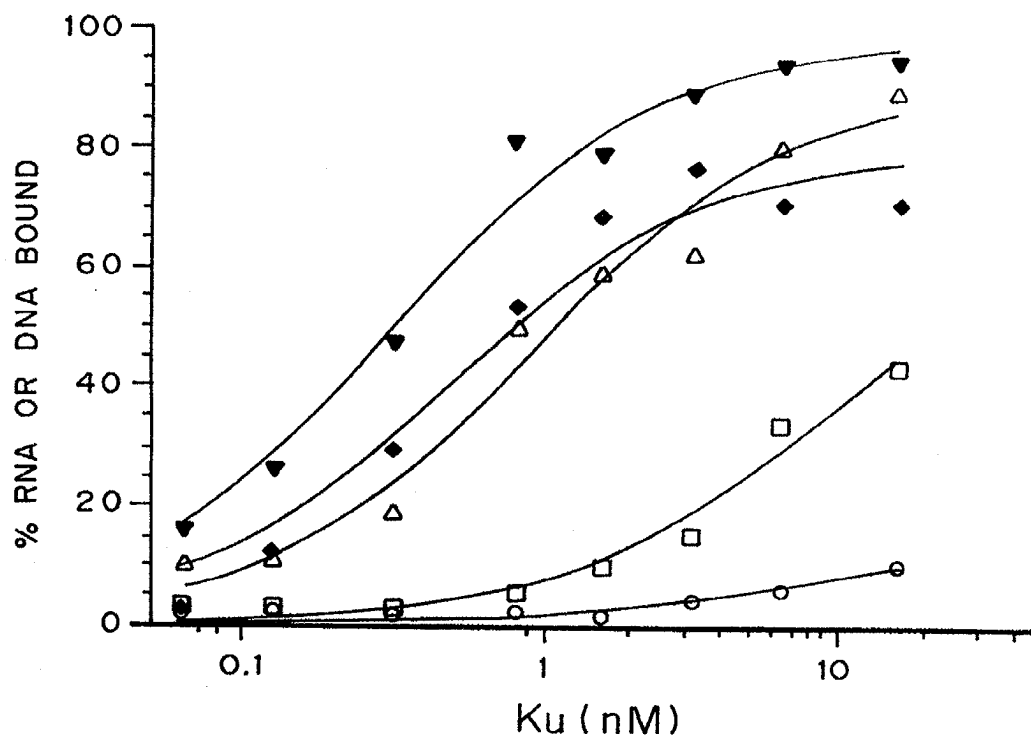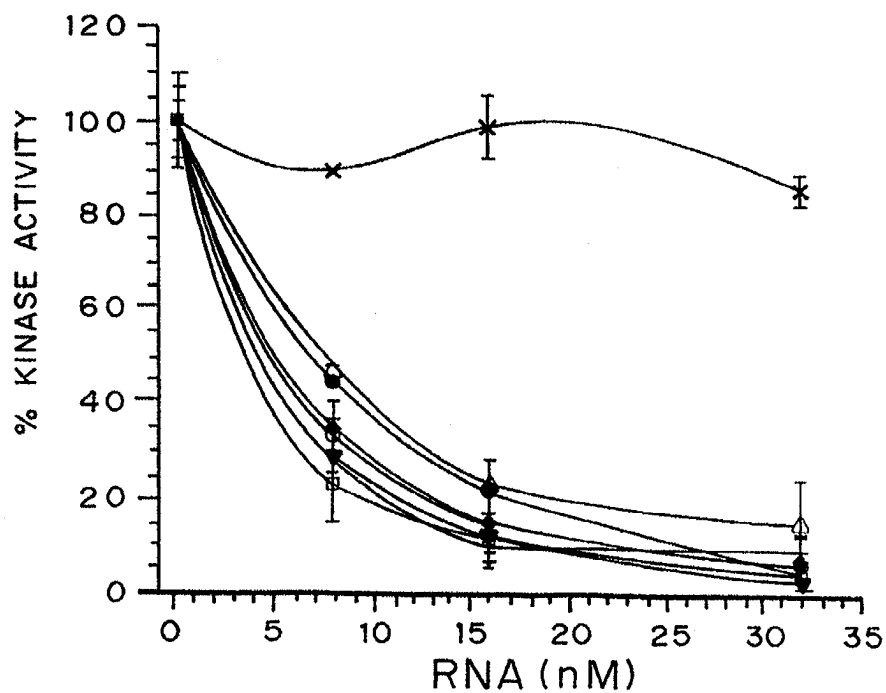
FIG. 5

FIG. 6A

FIG. 6B

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C12N15/11   C12Q1/68   C07H21/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C12N   C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KACZMARSKI W ET AL: "LUPUS AUTOANTIGEN KU PROTEIN BINDS HIV-1 TAR RNA IN VITRO" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 196, no. 2, pages 935-942, XP002061470 ISSN: 0006-291X cited in the application the whole document ——— <center>-/--</center>	1,2,4,5

[X] Further documents are listed in the continuation of box C.   [X]   Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 June 1999	17/06/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Andres, S

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	GIFFIN, W. ET AL.: "Sequence-specific DNA binding and transcription factor phosphorylation by Ku autoantigen/DNA-dependent protein kinase" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 272, 28 February 1997 (1997-02-28), pages 5647-5658, XP002105043 cited in the application page 5650, right-hand column, line 15 -, sentence 48 page 5652, left-hand column, line 4 -, sentence 19 page 5655, right-hand column, line 17 -, sentence 20	1,2,8,9
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A	WO 91 19813 A (UNIV COLORADO FOUNDATION) 26 December 1991 (1991-12-26) cited in the application page 8, line 33 - page 12, line 4 page 54, line 24 - page 56, line 28 claims figure 22	
A	BLISS, T. & LANE, D.: "Ku selectively transfers between DNA molecules with homologous ends" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 272, 28 February 1997 (1997-02-28), pages 5765-5773, XP002105045 cited in the application	

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	YOO S ET AL: "Characterization of the RNA binding properties of Ku protein." BIOCHEMISTRY, (1998 FEB 3) 37 (5) 1336-43., XP002105046 the whole document	1-9
T	DYNAN W S ET AL: "Interaction of Ku protein and DNA - dependent protein kinase catalytic subunit with nucleic acids." NUCLEIC ACIDS RESEARCH, (1998 APR 1) 26 (7) 1551-9., XP002105047	

1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/ 27728

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

   Remark: Although claims 8 to 14 (as far as in vivo methods are concerned)
   are directed to a method of treatment of the human/animal body,
   the search has been carried out and based on the alleged effects
   of the compound/composition.

2. [ ] Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such
   an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all
   searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report
   covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   [ ] The additional search fees were accompanied by the applicant's protest.

                               [ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

INTERNATIONAL SEARCH REPORT

Information on patent family members

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